

EVALUATION OF GLYPHOSATE TOLERANCE AND INFLUENCE OF PLANT GROWTH
STAGE AND TEMPERATURE ON GLYPHOSATE EFFICACY IN COMMON
LAMBSQUARTERS (*CHENOPODIUM ALBUM* L.)

by

RANDY D DEGREEFF

B.S., North Dakota State University, 2010

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2015

Approved by:

Co-Major Professor
Mithila Jugulam

Co-Major Professor
J. Anita Dille

Copyright

RANDY D DEGREEFF

2015

Abstract

Common lambsquarters (*Chenopodium album* L.) is an annual broadleaf weed species that competes with more than 40 crop species around the world. A biotype of common lambsquarters in north central KS (DK) was not controlled by a glyphosate application. Plant growth stage and temperature have been related to overall glyphosate efficacy on common lambsquarters. The objectives were to determine the: 1) tolerance of two biotypes of common lambsquarters to glyphosate, 2) efficacy of glyphosate on common lambsquarters at different growth stages, and 3) efficacy of glyphosate on common lambsquarters when grown at different temperatures. Greenhouse dose-response experiments, shikimate accumulation assay, and glyphosate uptake and translocation experiments were conducted using DK biotype and a known susceptible biotype (RL) of common lambsquarters for comparison. Dose-response results indicated elevated tolerance of the DK biotype to glyphosate based on the GR₅₀ (a dose causing 50% biomass reduction) values (373 g ae/ha for RL vs. 552 g ae/ha for DK). Similarly, the DK biotype accumulated slightly less shikimate in the leaf discs compared to the RL biotype. Minimal differences were observed in ¹⁴C-glyphosate uptake and translocation between the two biotypes. Greenhouse-grown common lambsquarters were treated with glyphosate at a field dose (1x) of 840 g ae/ha when they were 5-7, 10-12, 15-17 or 19-21 cm tall. Common lambsquarters were also grown in growth chambers for 1 wk maintained at d/n temperatures of 25/15, 32.5/22.5, or 40/30 C and then treated with 0-, 0.125-, 0.25-, 0.5-, 0.75, 1.0-, and 2.0-x rates of glyphosate at 8-10 cm tall. Visual injury was recorded 1 WAT and biomass was determined 2 WAT. Common lambsquarters plants treated at 5-7 cm were more susceptible than larger plants to glyphosate. Furthermore, plants were more susceptible to glyphosate when grown under lower temperatures of 25/15 C than higher temperatures. Overall, these results suggest that the DK biotype of

common lambsquarters appears to have elevated tolerance to glyphosate. Additionally, glyphosate should be applied early in the season when plants are small and temperatures are cooler for optimal control of common lambsquarters.

Table of Contents

List of Figures	vii
List of Tables	ix
Acknowledgements.....	x
Chapter 1 – Review of Literature.....	1
Common Lambsquarters - A Problem Weed in the United States	1
Herbicide Resistance in Common Lambsquarters	4
Evolution of Glyphosate Resistance in Weeds	6
Mechanisms of Glyphosate Resistance in Weeds.....	7
Response of Common Lambsquarters to Glyphosate.....	8
Uptake and Translocation of Glyphosate in Common Lambsquarters	9
Common Lambsquarters Growth Stage and Glyphosate Efficacy	10
Effect of Temperature on Common Lambsquarters Growth and Glyphosate Efficacy.....	11
Literature Cited	13
Chapter 2 – Evaluation of Glyphosate Tolerance and Influence of Plant Growth Stage and	
Temperature on Glyphosate Efficacy in Common Lambsquarters	21
Introduction.....	23
Materials and Methods	25
Common Lambsquarters Biotypes.....	25
Glyphosate Dose-Response	26
Shikimate Accumulation Assay	27
Glyphosate Uptake and Translocation	28
Common Lambsquarters Growth Stage and Glyphosate Efficacy	29
Common Lambsquarters Growth Temperature and Effect on Glyphosate Efficacy	29
Results.....	30
Glyphosate Dose-Response	30
Shikimate Accumulation Assay	30
Glyphosate Uptake and Translocation	31
Common Lambsquarters Growth Stage and Glyphosate Efficacy	31

Common Lambsquarters Growth Temperature Effect on Glyphosate Efficacy..... 32
Discussion..... 33
Literature Cited..... 37

List of Figures

Figure 1.1 Shikimate assay pathway disrupted by glyphosate	20
Figure 2.1 Whole-plant dose-response of DK and RL biotypes of common lambsquarters. The lines represent response curves and were generated using three-parameter log logistic regression model (Equation 2.1). GR ₅₀ for DK of 552 g ae ha ⁻¹ and RL of 373 g ae ha ⁻¹ . Nearly 20% of DK biotype survived 1680 g ae ha ⁻¹ glyphosate at 3 WAT but was controlled with a field rate (840 ga ae ha ⁻¹). Symbols represent means of aboveground dry biomass (% of treated)	41
Figure 2.2 Boxplot showing shikimate accumulation (100 µM glyphosate) in RL and DK biotypes of common lambsquarters. The tails represent minimum and maximum values. The boxes represent the 50 th percentile of those values. The solid line represents the median (most frequent) observation and the small square point is the mean observation. RL biotype accumulated 14.9 ng/uL of shikimate. DK biotype accumulated 19.2 ng/uL of shikimate..	42
Figure 2.3 Biomass (g/plant) of RL biotype of common lambsquarters 2 WAT for treated and untreated plants. Bars represent treated and untreated plant dry weight of means. Error bars are ± SE of two replicates. Percentages represent the difference in dry biomass between the treated and untreated plants at each plant height	43
Figure 2.4 Biomass (g/plant) of DK biotype of common lambsquarters 2 WAT for treated and untreated plants. Bars represent treated and untreated plant dry weight of means. Error bars are ± SE of two replicates. Percentages represent the difference in dry biomass between the treated and untreated plants at each plant height	44
Figure 2.5 Photographs of RL (top) and DK (bottom) biotype of common lambsquarters 2 WAT untreated (left) and treated (right) with glyphosate at 1x rate.....	45
Figure 2.6 Effect of growth temperature and glyphosate efficacy visual injury RL (top) and DK (bottom) 1 WAT at day/night low (LT) 25/15°C, medium (MT) 32.5/22.5°C, and high (HT) 40/30°C temperatures. Lines represent means ± SE of two replicates. 0% equals no injury, 100% equals plant death. Data were analyzed using the R version 3.1.1 Model 1	46
Figure 2.7 Photographs of common lambsquarters RL (left) and DK (right) plants grown at low (LT), medium (MT), and high (HT) temperatures at 2 WAT. Plants in each photograph (left to right): untreated, 105, 210, 420, 630 and 840 g ae/ha glyphosate rates.....	47

Figure 2.8 RL common lambsquarters biomass at 2 WAT in response to glyphosate as compared to untreated when grown under three temperature conditions: LT (25/15 C day/night), MT (32.5/22.5 C day/night), and HT (40/30 C day/night). Lines represent means \pm SE of two replicates. Percentages represent the difference in dry biomass between the treated and untreated plants at each plant temperature 48

Figure 2.9 DK common lambsquarters biomass at 2 WAT in response to glyphosate as compared to untreated when grown under three temperature conditions: LT (25/15 C day/night), MT (32.5/22.5 C day/night), and HT (40/30 C day/night). Lines represent means \pm SE of two replicates. Percentages represent the difference in dry biomass between the treated and untreated plants at each plant temperature 49

List of Tables

Table 2.1 Distribution of ¹⁴ C-labeled glyphosate in RL and DK biotypes of common lambsquarters	50
Table 2.2 Visual injury for plant growth heights of RL and DK biotypes of common lambsquarters	51

Acknowledgements

I would like to extend my sincerest gratitude to my major professors, Dr. Mithila Jugulam and Dr. J. Anita Dille for their extensive support, guidance, and encouragement throughout my entire program. I would also like to thank Dr. Dallas Peterson for his time and input serving as my committee member. I would like to thank the Department of Agronomy at Kansas State University for providing the funding and research materials for this research. Finally, I would like to thank all of my professors who have helped to increase my knowledge by sharing their time, talent, and expertise. I truly appreciate everything each one of you has done to advance my education.

I would also like to extend my thanks to my fellow graduate students along with Amar Godar, Junjun Ou, and Vijaya Varanasi for their friendship, camaraderie, support, and encouragement throughout my graduate experience. I would like to thank my entire family including my parents, siblings, in-laws, and children (Joshua, Lacey, Alida, and Hunter) for believing in me and supporting me through the ups and downs. Finally, I would like to thank my wife, Becky, for absolutely everything.

Chapter 1 - Review of Literature

Common Lambsquarters – A Problem Weed in the United States

Common lambsquarters (*Chenopodium album* L.) is an annual broadleaf weed species that originated in Europe and Asia. Common lambsquarters is considered one of the most important weeds in agriculture, ranking in the top five most widely distributed weeds competing with more than 40 crop species around the world (Holm et al., 1977). It is also considered as a principle weed by corn and soybean producers in the United States. Crop yield loss due to common lambsquarters competition can be considerable. Soybean yield loss of 20% occurred when postemergence herbicides were not applied prior to five weeks after crop emergence (Crook & Renner, 1990) and a 15% soybean yield loss occurred with a density of 16 weeds/10-m of row (Shurtleff & Coble, 1985). Corn yield loss of 12% occurred with only 4.9 common lambsquarters plants/m of row (Beckett et al., 1988) and 19 to 29% due to competition in field studies conducted in Quebec, Canada (Ngouajio et al., 1999). Other field experiments examined the interference of common lambsquarters with field corn at seven locations over a two-year period. Results varied from 0 to 100% yield loss in corn (Fischer et al., 2004). Significant sugarbeet root yield loss of 48% has also been found due to interference of common lambsquarters densities of 24 plants/30 m of row in field studies conducted at Fort Collins, CO (Schweizer, 1983).

Common lambsquarters is a summer annual and a member of the goosefoot family (*Chenopodiaceae*). It can emerge throughout the summer with majority of its emergence in mid- to late spring. Emergence studies conducted in the northeastern US found common lambsquarters have a long duration of emergence, reaching 50% completion by 475 degree days

(DD) and 2,225 DD for 95% completion from April through August. Complete emergence occurred anywhere from late July in the more southern US locations and into August in the more northern US locations (Myers et al., 2004). An emergence study conducted in Iowa based on soil temperature found 10% emergence at 19 growing degree days (GDD), 50% emergence at 153 GDD, and 90% emergence at 575 GDD with an observed extended emergence period from April to August (Werle et al., 2014). The prolonged emergence patterns suggest dormancy mechanisms likely play an important role in common lambsquarters (Myers et al., 2004).

Common lambsquarters is a distinctive, hearty, and competitive plant emerging in the spring. It generally flowers and sets seed in late summer and fall with the reproductive stage reached as soon as six weeks after emergence (Mohler & DiTommaso, 2006). The first true leaves are ovate-shaped and become distinctly alternate and may be purplish on the underside. Both surfaces are covered with a white granular substance (part of the scientific name *album*, Latin for white) (Curran et al., 2007). The leaves have a deep green to light green color that alternate, have petioles, and have no stipules. The stems are angular, branched, brownish-yellow and ridged with green or reddish parallel stripes. The stems are grooved, can be green or reddish in color, and can be smooth or hairless. Mature plants generally grow to a height of 0.61 to 1.82 m (2 to 6 ft). Plants are monoecious with perfect flowers that are clustered in contiguous glomerules. The flowers have five stamens and rarely have three or four. Each stamen possesses three to four anthers (Bassett & Crompton, 1978). The flowers can produce approximately 72,450 smooth, shiny seeds on an average-sized plant (Stevens, 1932) which are covered with a thin, papery film (Curran et al., 2007). Most of the seeds are black but about 3% of the seeds produced are also brown. The black seeds are more dormant and can remain viable for several decades (Curran et al., 2007). High percentages of black seeds are produced under longer day

length (Henson, 1970) and experience different germination responses to the ratio of red to far-red light. The seeds produced under longer day length are smaller and have a thicker testa than the less common brown seeds. The non-dormant brown seeds are produced under shorter days. Dormancy contributes to the success of common lambsquarters (Cumming, 1963).

Seeds are dispersed by various means including animal and equipment transfer (Curran et al., 2007). Common lambsquarters have been found to be dispersed from feces of cattle, pigs, sheep, and sparrows (Salisbury, 1961) and by agricultural practices, road construction, and moving of dirt and debris (Bassett & Crompton, 1978). Approximately 10 to 30% of a season's seed will germinate the following year (Forcella et al., 1992). Germination is encouraged by the presence of nitrate in the soil along with light and determinant day and night temperature fluctuations. The optimum seed depth for emergence is about 0.25 cm with very few seedlings emerging from greater than 2.54 cm soil depth.

Interference of common lambsquarters in corn and soybean cropping systems can vary depending upon emergence and growth rate of the weed. Mulugeta and Stoltenberg (1997) found common lambsquarters emergence increased 6-fold in tilled soils as opposed to no-till systems. Fischer et al., (2004) found competition for nutrients, moisture, and light with corn and soybean in the Midwest varied due to early emergence, rapid growth rate, and weed density relative to crop emergence and the environmental conditions including temperature and rainfall. Wet weather slowing emergence and/or early-season common lambsquarters growth were speculated as the cause of delayed emergence and reduced vigor of common lambsquarters in Illinois and Minnesota plot locations in 1996 examining corn yield loss due to common lambsquarters density resulting in limited to no competition. Conditions more favorable for earlier emergence would increase biomass of common lambsquarters and result in higher rates of yield loss

(Fischer et al., 2004). Beyond competition, common lambsquarters is also a host for several crop diseases including various mosaic viruses. Recommendations for common lambsquarters control are with mechanical cultivation and herbicide applications. Bromoxynil, dicamba and thifensulfuron can be used to control common lambsquarters post emergence in corn (Fielding & Stoller, 1990; Fuerst et al., 1986; Gentsch & Weber, 1984; Hagood, 1989; Malik, 1990).

Herbicide Resistance in Common Lambsquarters

Herbicide resistance in common lambsquarters has been a problem for many years. According to the International Survey of Herbicide Resistant Weeds (Heap, 2015), the first documented case of herbicide resistance in common lambsquarters was to atrazine and occurred in 1973 in Ontario, Canada in corn and cropland (Bandeem & McLaren, 1976). The first documented cases of ALS resistant common lambsquarters were in 2001 in Ontario, Canada, Michigan, and Ohio. Today there are 47 documented cases globally of herbicide resistance in common lambsquarters, include resistance to atrazine, metamiltron, metribuzin, thifensulfuron-methyl, tribenuron-methyl, cyanazine, lenacil, prometon, simazine, terbuthylazine, terbutryn, dicamba, linuron, imazethapyr, nicosulfuron, imazamox, and terbacil (Heap, 2015). In Pennsylvania, samples of common lambsquarters juvenile plants from 58 farms were sprayed with 0.45 kg ai/ha atrazine. Surviving plants were then sprayed with 1.03 kg ai/ha atrazine. On average, common lambsquarters were found to be 81% resistant to both rates. Common lambsquarters from four of the 58 farms were 9% or less triazine-susceptible, while common lambsquarters from 34 farms were greater than 90% triazine-resistant. The remaining 20 farms were found to have mixed populations of susceptible and resistant common lambsquarters (Bravo & Curran, 2001). Furthermore, in New Zealand common lambsquarters was the

country's first reported herbicide-resistant weed, found to be resistant to atrazine (Rahman, 1982).

Triazine-resistant common lambsquarters have been satisfactorily controlled with preemergence herbicides; pendimethalin provided the greatest control (98%) with acetochlor (86%) and metolachlor (66%) providing less consistent control in field studies conducted in the southwest Michigan from 1995 through 2000 (Chomas & Kells, 2004). Isoxaflutole, flumetsulam, and rimsulfuron plus thifensulfuron were found to provide 98% or greater control of triazine-resistant common lambsquarters in corn applied as preemergence products (Chomas & Kells, 2004). Resistance to ALS-inhibiting herbicides, such as those in the imidazolinone and sulfonyleurea families was documented in common lambsquarters from Michigan (Gower & Penner, 2002). Resistance in this population evolved as a result of at least 14 consecutive annual applications of ALS-inhibiting herbicides. Foliar-applications of imazamox provided 26% control, whereas, only 19% control was achieved with thifensulfuron when the resistant population was treated with 2X field rates of these herbicides compared to 90 and 93% control of a susceptible population (Gower & Penner, 2002).

A population of common lambsquarters was also found to be resistant to dicamba in several corn fields in New Zealand (James & Rahman, 2005). Recently Rahman et al. (2014) conducted greenhouse experiments with seeds collected from plants that survived field applications of dicamba. Results showed the plants could tolerate ≤ 1.2 kg/ha, which is four fold the recommended rate. Field experiments found the resistant population tolerated dicamba at ≤ 2.4 kg/ha, which is eight fold the recommended rate (Rahman et al., 2014). To date, there has been no documented resistance of glyphosate in common lambsquarters; however, elevated

tolerance to glyphosate has been found in several locations (Hite et al., 2008; Kniss et al., 2007; Sivesind et al., 2011; Westhoven et al., 2008b).

Evolution of Glyphosate Resistance in Weeds

Glyphosate is used world-wide due to its ability to economically control a broad spectrum of weeds. Roundup™ was the first glyphosate herbicide introduced by Monsanto in 1974. The systemic nature of glyphosate allows for good control of perennial weeds. In a review of 630 papers published in scientific journals between mid-1995 and mid-1998, Baylis (2000) found that one weakness of glyphosate is the need for higher rates to control the more tolerant broadleaved weeds. Further, a lack of soil activity results in no residual activity and no control of weeds emerging after application (Baylis, 2000). Glyphosate is an inhibitor of the chloroplast enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Inhibiting EPSPS activity disrupts the shikimate pathway, inhibiting aromatic amino acid production and causing plant death (Figure 1.1). A contributing factor to the evolution of glyphosate-resistant weeds has been extensive and exclusive use of glyphosate upon introduction of transgenic glyphosate-resistant crops including soybeans, corn, cotton, and canola (Powles & Yu, 2010).

Currently there are 32 different weed species with evolved resistance to glyphosate worldwide with 14 known to be resistant in the United States (Heap, 2015). Horseweed (*Conyza canadensis*) was the first glyphosate-resistant weed documented in the US (VanGessel, 2001). Weeds found to be resistant to glyphosate in Kansas include horseweed, waterhemp (*Amaranthus rudis*), giant ragweed (*Ambrosia trifida*), common ragweed (*Ambrosia artemisiifolia*), kochia (*Kochia scoparia*), and Palmer amaranth (*Amaranthus palmeri*) (Heap, 2015).

The likelihood of a weed species to evolve resistance to glyphosate is dependent on various factors, including the weed biology, and frequency and use rate of glyphosate. An increased intensity in the use of glyphosate can increase the number of resistant plants within a population (Boerboom & Owen, 2006). Resistance can be caused by an altered target site (Wakelin & Preston, 2006), metabolism (Cobb & Reade, 2010), overexpression of the target enzyme (Powles & Yu, 2010), gene duplication, active vacuole sequestration, limited cellular uptake and a rapid necrosis response (Sammons & Gaines, 2014). Other factors that may reduce the performance of glyphosate in a weed population include: incorrect application rate for weed size or species, rain before glyphosate is completely absorbed by the weed, weather-stressed weeds (drought, cold, etc.), incomplete spray coverage of weeds below the crop canopy, reduced glyphosate activity with early morning, late evening, or night applications, and weeds emerging after glyphosate application (Boerboom & Owen, 2006).

Mechanisms of Glyphosate Resistance in Weeds

Sammons et al. (2007) catalogued the basic resistance mechanisms as target-site resistance, which is an amino acid substitution that affects herbicide interaction at the target enzyme; metabolism, which is a chemical modification of the herbicide by either conjugation or degradation; and exclusion of the herbicide from the target, physically or with enhanced cuticular and structural barriers or physiologically with active transporters. In a recent review of research on weed resistance to glyphosate, Sammons and Gaines (2014) noted mechanisms of glyphosate resistance in weeds included exclusion mechanisms of vacuolar sequestration and reduced translocation; EPSPS gene duplication; and EPSPS target-site mutations. Vacuolar sequestration rates between glyphosate resistant and glyphosate susceptible *C. canadensis* was found to be 10x higher in the resistant population (Ge et al., 2013). Reduced translocation occurs in glyphosate

resistant weeds likely due to the rapid necrosis and the inability of glyphosate to be exported from mature plant leaves (Sammons & Gaines, 2014). Gene duplication is the heritable replication of a DNA segment resulting in additional gene copies within the genome (Innan & Kondrashov, 2010). Examples of EPSPS gene duplication has been reported in glyphosate resistant cell lines. Multiple studies have shown the glyphosate resistance levels appear to increase with higher EPSPS genomic copy number (Sammons & Gaines, 2014). EPSPS target-site mutations occur in glyphosate resistant weeds changing the hydrophobic Pro106 amino acid of the EPSPS to the hydrophobic amino acids Ala or Leu, or the hydrophilic amino acids Ser and Thr (Sammons & Gaines, 2014).

Response of Common Lambsquarters to Glyphosate

Recently in the United States, some accessions of common lambsquarters have shown inconsistent response to glyphosate (Hite et al., 2008; Kniss et al., 2007; Schuster et al., 2007; Sivesind et al., 2011; Westhoven et al., 2008b) implying tolerance to glyphosate. According to the WSSA (1998), tolerance is “the inherent ability of a species to survive and reproduce after herbicide treatment.” This suggests that there was no selection or genetic manipulation involved in this species to tolerate glyphosate treatment. Producers have been reporting problems controlling common lambsquarters with postemergence glyphosate applications. Anecdotal observations in several states have suggested that common lambsquarters biotypes are not being controlled with glyphosate in Roundup Ready[®] crops (Owen & Zelaya, 2005). It has also been noted that the size of common lambsquarters at the time of application influences the plant response to glyphosate (Curran et al., 2007). Common lambsquarters possesses a high level of genetic variability, making it possible that a given population will contain various biotypes exhibiting different levels of glyphosate susceptibility (King et al., 2004).

A population of common lambsquarters from Virginia survived a glyphosate rate of 454 g ae/ha, with the more tolerant trait passed on to the next generation (King et al, 2004). Curran et al. (2007) stated that weed scientists in Ohio and Indiana have identified a common lambsquarters biotype that appears to have low-level glyphosate resistance in at least a dozen fields. Hite et al. (2008) reported the first case of differential response of glyphosate sensitivity between two common lambsquarters biotypes from Virginia, finding vigor reduction of 73 and 97% between the two biotypes 28 DAT. Two years later, the two biotypes showed reduced vigor of 66 and 89%, showing an increase in tolerance in the F2 plants. Previously, ten out of 13 populations of common lambsquarters collected in 2005 and 2006 in Indiana showed enhanced tolerance to glyphosate compared to a known susceptible population (Westhoven et al., 2008a). In a greenhouse and field dose-response study, Westhoven et al. (2008b) found four biotypes that were 2.6- to 7.8-fold (greenhouse study) and 1.67- to 2.77-fold (field study) more tolerant to glyphosate than the susceptible biotype. The tolerant biotypes were found to be taller, with more leaf area, and also produced a greater amount of dry biomass compared to the susceptible biotype. The tolerant biotypes also advanced through the growth stages more rapidly and initiated flower primordia four weeks earlier than the sensitive biotypes during the early growing season, but produced less biomass at plant maturity (Westhoven et al., 2008b).

Uptake and Translocation of Glyphosate in Common Lambsquarters

Growth chamber experiments conducted on a common lambsquarters population from Nebraska showed greater than 75% of the radioactivity absorbed remained in the glyphosate-treated leaf in all plants at all heights (Schuster et al., 2007). Translocation of glyphosate was more rapid and extensive in 15-cm plants than in 2.5-cm plants, which could be attributed to a

larger carbohydrate sink in the more developed plants (Chachalis et al., 2001; Devine et al., 1993; Hennigh et al., 2005; Schuster et al., 2007).

Absorption, translocation, and exudation studies were conducted using glyphosate-tolerant (T) common lambsquarters from Indiana and susceptible (S) plants from Wisconsin. Translocation of ^{14}C -glyphosate was greater above the treated leaf in the S accession than the T accession, suggesting an important role of reduced translocation in conferring tolerance of common lambsquarters to glyphosate. The ED_{50} value for the T accession was eight fold greater than that of the S accession based on 95% confidence intervals (Yerka et al., 2013).

Common Lambsquarters Growth Stage and Glyphosate Efficacy

Previous research showed that plants are most susceptible to herbicides at early growth stages (Coetzer et al., 2002; Krausz et al., 1996). This may be due to the fact that young and rapidly growing plants will absorb more herbicide than mature plants (Wanamarta & Penner, 1989). Schuster et al. (2007) conducted dose response studies in a greenhouse on common lambsquarters collected from Ohio, Nebraska, North Dakota, and Kansas. Seedlings were treated with various rates of glyphosate, i.e. 0, 0.125, 0.25, 0.5, 1, 2, 4, and 8 times the typical use rate of 1.1 kg ae/ha at different plant heights of approximately 2.5, 7.5, or 15 cm tall. Visual injury was assessed at 1, 2, and 3 weeks after treatment (WAT). The results indicated that early seedling stages of common lambsquarters were found to be susceptible to glyphosate; however, as the plants developed, the extent of tolerance varied. At least half of the 15-cm plants survived the highest rates of glyphosate. Growth after treatment was slow and was characterized by increased growth of lateral and basal branches. Symptoms generally peaked 2 WAT then plants either died or slowly recovered. Tolerance to glyphosate when applied to the 15-cm plants compared to the 2.5-cm plants increased 2.4-fold in Kansas biotypes and 5.5-fold in Ohio biotypes (Schuster et

al., 2007). The results were not surprising due to young plants being more metabolically active and generally more susceptible to glyphosate (Chachalis et al., 2001; Devine et al., 1993; Hennigh et al., 2005).

Schuster et al. (2007) speculated that the higher calcium content in more developed plants could contribute to less glyphosate injury, which supports the Hall et al. (2000) theory that reduced efficacy of glyphosate on certain species can be partly explained by the antagonistic interaction of the anionic form of glyphosate with cations such as calcium within the plant. Schuster et al. (2007) found 3X higher calcium content in 15-cm common lambsquarters than in 2.5-cm plants. The antagonistic effect of calcium on glyphosate activity has also been documented in previous research (Hall et al., 2000; Nalewaja & Matysiak 1993; Thelen et al., 1995).

Effect of Temperature on Common Lambsquarters Growth and Glyphosate Efficacy

Effect of temperature on overall efficacy of glyphosate in controlling a variety of plants is mixed. Previous research on a variety of weeds grown in controlled environments has shown cool, dry conditions result in reduced absorption, translocation, and overall efficacy of glyphosate (Dall'Armellina & Zimdahl, 1989; Jordan 1977; Klevorn & Wyse, 1984; Masiunas & Weller, 1988; Reddy 2000). Field studies from two research sites in Wyoming reported that differences in temperature and relative humidity might have contributed to large differences in mortality of common lambsquarters. The site with warmer, more humid environmental conditions, which are more favorable for glyphosate efficacy, showed high mortality from 840 g ae/ha regardless of treatment history while the site with drier and cooler environmental conditions showed mortality ranging from 0 to 100% with a peak near 50% (Kniss et al., 2007).

On the other hand, Sivesind et al. (2011) found no relationship between temperature and glyphosate efficacy on populations in Wisconsin; control was consistently high regardless of variations in the environmental conditions including rainfall after treatment, relative humidity at the time of treatment, and air temperatures before, during, and after application.

Glyphosate efficacy has been shown to vary based upon the growth stage of the plant at the time of application; however, research results regarding plant growth temperature at the time of glyphosate application are mixed. Therefore, the overall goal of this research was to understand glyphosate interactions with common lambsquarters. The specific objectives were to determine the:

1. tolerance of two biotypes of common lambsquarters to glyphosate as influenced by the uptake and translocation of glyphosate,
2. efficacy of glyphosate on common lambsquarters at different growth stages, and
3. efficacy of glyphosate on common lambsquarters when grown at different temperatures.

Literature Cited

- Bandeem JD, McLaren RD (1976). Resistance of *Chenopodium album* to triazine herbicides. Canadian Journal of Plant Science 56: 411-412.
- Bassett IJ, Crompton CW (1978). The biology of Canadian weeds. 32. *Chenopodium album* L. Canadian Journal of Plant Science 58:1061-1072
- Baylis AD (2000). Why glyphosate is a global herbicide: Strengths, weaknesses and prospects. Pest Management Science 56:299-308
- Beckett TH, Stoller EW, Wax LM (1988). Interference of four annual weeds in corn (*Zea mays*). Weed Science 36:764-769
- Boerboom C, Owen M (2006). Facts about glyphosate-resistant weeds. Glyphosate, weeds, and crops. Purdue Extension Education. GWC-1
- Bravo MA, Curran WS (2001). A survey of triazine resistant common lambsquarters on Pennsylvania farms. Northeast Weed Science Society Abstr 55:18
- Chachalis D, Reddy KN, Elmore DD, Steele L (2001). Herbicide efficacy, leaf structure, and spray droplet contact angle among *Ipomoea* species and smallflower morningglory. Weed Science 49:628-634
- Chomas AJ, Kells JJ (2004). Triazine-resistant common lambsquarters (*Chenopodium album*) control in corn with preemergence herbicides. Weed Technology 18:551-554
- Cobb AH, Reade PH (2010). *Herbicides and Plant Physiology*. Chichester, West Sussex: Wiley-Blackwell, Print
- Coetzer E, Al-Khatib K, Peterson DE (2002). Glufosinate efficacy on *Amaranthus* species in glufosinate-resistant soybean (*Glycine max*). Weed Technology 16:326-331

- Crook TM, Renner KA (1990). Common lambsquarters (*Chenopodium album*) competition and time of removal in soybeans (*Glycine max*). *Weed Science* 38:358-364
- Curran B, Sprague C, Stachler J, Loux M (2007). Biology and management of common lambsquarters. Glyphosate, weeds, and crops. Purdue Extension Education. GWC-11
- Cumming BG (1963). The dependence of germination of photoperiod, light quality and temperature in *Chenopodium*. *Canadian Journal of Botany* 41:1211-1233
- Dall'Armellina AA, Zimdahl RL (1989). Effect of watering frequency, drought, and glyphosate on growth of field bindweed (*Convolvulus arvensis*). *Weed Science* 37:314-318
- Devine MD, Duke SO, Fedtke C (1993). *Physiology of Herbicide Action*. Englewood Cliffs, NJ: Prentice Hall Press, Pp. 95-109
- Fielding RJ, Stoller EW (1990). Effects of additives on the efficacy, uptake, and translocation of methyl ester of thifensulfuron. *Weed Science* 38:172-178
- Fischer DW, Harvey RG, Bauman TT, Phillips S, Hart SE, Johnson GA, Kells JJ, Westra P, Lindquist JL (2004). Common lambsquarters (*Chenopodium album*) interference with corn across the northcentral United States. *Weed Science* 52:1034-1038
- Forcella F, Wilson RG, Renner KA, Dekker J, Harvey RG, Alm DA, Buhler DD, Cardina J (1992). Weed seed banks of the US corn belt: Magnitude, variation, emergence, and application. *Weed Science* 40:636-644
- Fuerst EP, Barrett M, Penner D (1986). Control of triazine-resistant common lambsquarters (*Chenopodium album*) and two pigweed species (*Amaranthus* spp) in corn (*Zea mays*). *Weed Science* 34:440-443
- Ge X, d'Avignon DA, Ackerman J, Ostrander E, Sammons RD (2013). Applications of ³¹P NMR spectroscopy to glyphosate studies in plants: Insights into cellular uptake and vacuolar

- sequestration correlated to herbicide resistance. In *Herbicides: Biological activity, classification and health and environmental implications*. Nova Science, Hauppauge, NY
- Gentsch BJ, Weber JB (1984). The efficacy of bromoxynil applied in irrigation water. *Southern Weed Science Society* 37:112-118
- Gower SA, Penner D (2002). Discovery of a common lambsquarters population resistant to ALS inhibitors in Michigan. *North Central Weed Science Society Abstract* 57:156
- Hagood ES (1989). Control of triazine-resistant smooth pigweed (*Amaranthus hybridus*) and common lambsquarters (*Chenopodium album*) in no-till corn (*Zea mays*). *Weed Technology* 3:136-142
- Hall CJ, Hart CA, Jones CA (2000). Plants as source of cations antagonistic to glyphosate activity. *Pest Management Science* 56:351-358
- Heap IM (2015). The international survey of herbicide resistant weeds. Online. Internet. January 15, 2015. Available www.weedscience.org.
- Hennigh DS, Al-Khatib K, Stahlman PW, Shoup DE (2005). Prairie cupgrass (*Eriochloa contracta*) and windmillgrass (*Chloris verticillata*) response to glyphosate and acetyl-CoA carboxylase-inhibiting herbicides. *Weed Science* 53:315-322
- Henson IE (1970). The effects of light, potassium nitrate and temperature on germination of *Chenopodium album*. *Weed Research* 10:27-39
- Hite GA, King SR, Hagood ES, Holtzman GI (2008). Differential response of a Virginia common lambsquarters (*Chenopodium album*) collection to glyphosate. *Weed Science* 56:203-209
- Holm LG, Plucknett DL, Pacho JV, Herberger HP (1977). The world's worst weeds distribution and biology. Honolulu, HI: Univ, Press of Hawaii. Pp. 84-91.

- Innan H, Kondrashov F (2010). The evolution of gene duplications: Classifying and distinguishing between models. *National Review of Genetics* 11:97-108
- James TK, Rahman A (2005). Fathen (*Chenopodium album*): a biotype resistant to dicamba. *New Zealand Plant Protection* 59:250-254
- Jordan TN (1977). Effects of temperature and relative humidity on the toxicity of glyphosate to bermudagrass (*Cynodon dactylon*). *Weed Science* 25:448-451
- King SR, Hagood ES, Westwood JH (2004). Differential response of common lambsquarters (*Chenopodium album*) biotype to glyphosate. *Weed Science Society of America Abstr* 44:68
- Klevron TB, Wyse DL (1984). Effect of soil temperature and moisture on glyphosate and photoassimilate distribution in quackgrass (*Agropyron repens*). *Weed Science* 32:402-407
- Kniss AR, Miller SD, Westra PH, Wilson RG (2007). Glyphosate susceptibility in common lambsquarters (*Chenopodium album*) is influenced by parental exposure. *Weed Science* 55:572-577
- Krausz RF, Kapusta G, Matthews JL (1996). Control of annual weeds with glyphosate. *Weed Technology* 10:957-962
- Malik N (1990). Weed control during establishment and yield response of timothy (*Phleum pretense*). *Weed Technology* 4:598-605
- Masiunas JB, Weller SC (1988). Glyphosate activity in potato (*Solanum tuberosum*) under different temperature regimes and light levels. *Weed Science* 36:137-140

- Mohler CI, DiTommaso A (2006). Common lambsquarters. *In* Manage Weeds on Your Farm: A Guide to Ecological Strategies. Sustainable Agriculture Research and Education (SARE). In prep.
- Mulugeta D, Stoltenberg DE (1997). Increased weed emergence and seed bank depletion by soil disturbance in a no-tillage system. *Weed Science* 45:234-241
- Myers MW, Curran WS, VanGessel MJ, Calvin DD, Mortensen DA, Majek BA, Karsten HD, Roth GW (2004). Predicting weed emergence for eight annual species in northeastern United States. *Weed Science* 52:913-919
- Nalewaja JD, Matysiak R (1993). Influence of diammonium sulfate and other salts on glyphosate phytotoxicity. *Pest Science* 38:77-84
- Ngouajio M, Lemieux C, Leroux GD. (1999). Prediction of corn (*Zea mays*) yield loss from early observations of the relative leaf area and the relative leaf cover of weeds. *Weed Science* 47:297-304
- Owen MDK, Zelaya IA (2005). Herbicide-resistant crops and weeds resistance to herbicides. *Pest Management Science* 61:301-311
- Powles SB, Yu Q (2010). Evolution in action: Plants resistant to herbicides. *Annual Review of Plant Biology* 61:317-347
- Rahman A (1982). Resistance of weeds to herbicides in New Zealand. *In Pesticide Resistance Seminar* (ed. By Geelen JA and Esson MJ). Hawkes Bay Branch of New Zealand Weed and Pest Control Society, Hastings, New Zealand, 13-16
- Rahman A, James T, Trollove M (2014). Characteristics and control of dicamba-resistant common lambsquarters (*Chenopodium album*). *Weed Biology and Management* 14:88-98

- Reddy KN (2000). Factors affecting toxicity, absorption, and translocation of glyphosate in redvine (*Brunnichia ovate*). *Weed Technology* 14:457-462
- Salisbury E (1961). *Weeds and aliens*. Collins Clear-type Press, London and Glasgow
- Sammons RD, Gaines TA (2014). Glyphosate resistance: state of knowledge. *Pest Management Science* 70:1367-1377
- Sammons RD, Heering DC, Dinicola N, Glick H, Elmore GA (2007). Sustainability and stewardship of glyphosate and glyphosate-resistant crops. *Weed Technology* 21:347-354.
- Schuster CL, Shoup DE, Al-Khatib K (2007) Response of common lambsquarters (*Chenopodium album*) to glyphosate as affected by growth stage. *Weed Science* 55:147-151
- Schweizer EE (1983). Common lambsquarters (*Chenopodium album*) interference in sugarbeets (*Beta vulgaris*). *Weed Science* 31:5-8
- Shurtleff JL, Coble HD (1985). Interference of certain broadleaf weed species in soybeans (*Glycine max*). *Weed Science* 33:654-657
- Sivesind EC, Gaska JM, Jeschke MR, Boerboom CM, Stoltenberg DE (2011). Common lambsquarters response to glyphosate across environments. *Weed Technology* 25:44-50
- Stevens OA (1932). The number and weight of seeds produced by weeds. *American Journal of Botany* 19:784-794
- Thelen KD, Jackson EP, Penner D (1995). The basis for the hard-water antagonism of glyphosate activity. *Weed Science* 43:541-548
- VanGessel MJ (2001). Glyphosate-resistant horseweed from Delaware. *Weed Science* 49:703-705

- Wakelin AM, Preston C (2006). Target site mutation is present in glyphosate-resistant *Lolium rigidum* population. *Weed Research* 46:432-440
- Wanamarta G, Penner D (1989). Foliar absorption of herbicides. *Review of Weed Science* 4:215-231
- [WSSA] Weed Science Society of America (1998). Herbicide resistance and herbicide tolerance defined. *Weed Technology* 12:789-790
- Werle R, Sandell LD, Buhler DD, Hartzler RG, Lindquist JL (2014). Predicting emergence of 23 summer annual weed species. *Weed Science* 62:267-279
- Westhoven AM, Davis VM, Gibson KD, Weller SC, Johnson WG (2008a). Field presence of glyphosate-resistant horseweed (*Conyza Canadensis*), common lambsquarters (*Chenopodium Album*), and giant ragweed (*Ambrosia Trifida*). *Weed Technology* 22:544-548
- Westhoven AM, Kruger GR, Gerber CK, Stachler JM, Loux MM, Johnson WG (2008b). Characterization of selected common lambsquarters (*Chenopodium album*) biotypes with tolerance to glyphosate. *Weed Science* 56:685-691
- Yerka MK, Wiersma AT, Lindenmayer RB, Westra P, Johnson WG, de Leon N, Stoltenberg DE (2013). Reduced translocation is associated with tolerance of common lambsquarters (*Chenopodium album*) to glyphosate. *Weed Science* 61:353-360

SHIKIMATE PATHWAY

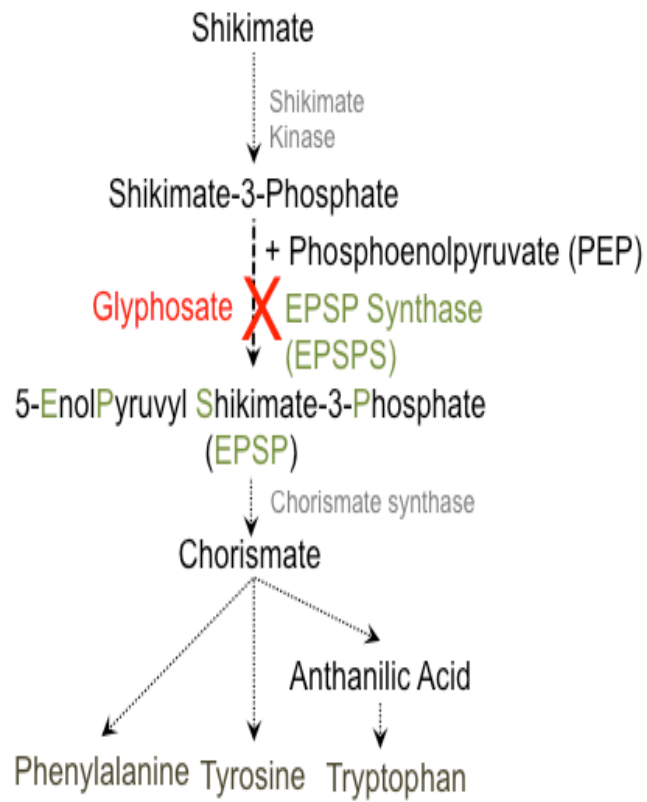


Figure 1.1 Shikimate assay pathway disrupted by glyphosate

Chapter 2 - Evaluation of Glyphosate Tolerance and Influence of Plant Growth Stage and Temperature on Glyphosate Efficacy in Common Lambsquarters (*Chenopodium album* L.)

Abstract

Common lambsquarters (*Chenopodium album* L.) is an annual broadleaf weed species that competes with more than 40 crop species around the world. A biotype of common lambsquarters in north central KS (DK) was not controlled by a glyphosate application. Plant growth stage and temperature have been related to overall glyphosate efficacy on common lambsquarters. The objectives were to determine the: 1) tolerance of two biotypes of common lambsquarters to glyphosate, 2) efficacy of glyphosate on common lambsquarters at different growth stages, and 3) efficacy of glyphosate on common lambsquarters when grown at different temperatures. Greenhouse dose-response experiments, shikimate accumulation assay, and glyphosate uptake and translocation experiments were conducted using DK biotype and a known susceptible biotype (RL) of common lambsquarters for comparison. Dose-response results indicated elevated tolerance of the DK biotype to glyphosate based on the GR₅₀ (a dose causing 50% biomass reduction) values (373 g ae/ha for RL vs. 552 g ae/ha for DK). Similarly, the DK biotype accumulated slightly less shikimate in the leaf discs compared to the RL biotype. Minimal differences were observed in ¹⁴C-glyphosate uptake and translocation between the two biotypes. Greenhouse-grown common lambsquarters were treated with glyphosate at a field dose (1x) of 840 g ae/ha when they were 5-7, 10-12, 15-17 or 19-21 cm tall. Common lambsquarters were also grown in growth chambers for 1 wk maintained at d/n temperatures of 25/15, 32.5/22.5, or 40/30 C and then treated with 0-, 0.125-, 0.25-, 0.5-, 0.75, 1.0-, and 2.0-x rates of glyphosate at 8-10 cm tall. Visual injury was recorded 1 WAT and biomass was determined 2 WAT. Common

lambsquarters plants treated at 5-7 cm were more susceptible than larger plants to glyphosate. Furthermore, plants were more susceptible to glyphosate when grown under lower temperatures of 25/15 C than higher temperatures. Overall, these results suggest that the DK biotype of common lambsquarters appears to have elevated tolerance to glyphosate. Additionally, glyphosate should be applied early in the season when plants are small and temperatures are cooler for optimal control of common lambsquarters.

Introduction

Common lambsquarters is a principle broadleaf weed species in corn and soybean cropping systems in the United States. Common lambsquarters has contributed to yield loss in soybean, corn, and sugarbeet (Beckett et al., 1988; Crook & Renner, 1990; Shurtleff & Coble, 1985; Schweizer, 1983). Biological characteristics of common lambsquarters including prolonged emergence patterns spanning from April through August (Myers et al., 2004) and the ability for seeds to remain dormant for several decades (Curran et al., 2007) both contribute to making it a highly problematic competitive weed. An average-sized plant can produce approximately 72,450 seeds (Stevens, 1932). Common lambsquarters can be controlled by mechanical cultivation and herbicide applications. Bromoxynil, dicamba and thifensulfuron can be used to control common lambsquarters postemergence in corn (Fielding & Stoller, 1990; Fuerst et al., 1986; Gentsch & Weber, 1984; Hagood, 1989; Malik, 1990).

Glyphosate, a widely used herbicide in agriculture, was first introduced by Monsanto in 1974 for broad spectrum weed control (Baylis, 2000). Frequent and continuous use of glyphosate has resulted in weed tolerance or resistance to this herbicide. Currently there are 32 different weed species with evolved resistance to glyphosate worldwide with 14 species known to be resistant in the US (Heap, 2015). Glyphosate is an inhibitor of the chloroplast enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Inhibiting EPSPS activity disrupts the shikimate pathway resulting in depletion of aromatic amino acid production causing plant death (Jaworski, 1972). Recently in the United States some accessions of common lambsquarters have shown inconsistent response to glyphosate (Hite et al., 2008; Kniss et al., 2007; Sivesind et al., 2011; Westhoven et al., 2008) and other postemergence herbicides. Environmental conditions before, during, and after foliar application along with the size of common lambsquarters at the

time of application influence the plant response to glyphosate (Curran et al., 2007). In general glyphosate-tolerant common lambsquarters biotypes collected from four counties in Indiana were taller and had more leaf area and biomass than susceptible plants. They also advanced through the growth stages more rapidly than susceptible biotypes (Westhoven et al., 2008).

The Weed Science Society of America (WSSA) (1998) defines herbicide tolerance as “the inherent ability of a species to survive and reproduce after herbicide treatment” and herbicide resistance as “the inherited ability to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type.” Common lambsquarters biotypes were found tolerant to glyphosate in several states in the US (King et al, 2004; Curran et al., 2007; Hite et al., 2008; Westhoven et al., 2008). However, level of tolerance to glyphosate varies based on plant growth stage and temperature at time of application. Absorption, translocation, and exudation studies conducted on glyphosate-tolerant common lambsquarters from Indiana suggest that the tolerant (T) plants translocated less glyphosate to above-treated area compared to susceptible sensitive biotypes (Yerka et al., 2013). Furthermore, the ED₅₀ value for the T accession was eight fold greater than that of the susceptible accession based on 95% confidence intervals (Yerka et al., 2013). Tolerance to glyphosate was increased 2.4-fold in Kansas biotypes and 5.5-fold in Ohio biotypes when glyphosate was applied to 15-cm plants compared to 2.5-cm plants (Schuster et al., 2007).

Research results that examined the effect of temperature on overall efficacy of glyphosate have been mixed. Earlier research on a variety of weeds has shown cool, dry conditions result in reduced efficacy of glyphosate (Dall’Armellina & Zimdahl, 1989; Jordan, 1977; Klevorn & Wyse, 1984; Masiunas & Weller, 1988; Reddy, 2000). Field studies conducted in Wyoming found warmer and humid environments are more favorable to improve glyphosate efficacy

(Kniss et al., 2007). On the other hand, Sivesind et al. (2011) suggested that the control of common lambsquarters by glyphosate was consistently high regardless of the environmental conditions in Wisconsin including rainfall after treatment, relative humidity at the time of treatment, and air temperatures before, during, and after application. Increased levels of tolerance and glyphosate efficacy have varied based upon the growth stage of the plant at the time of application; however, research results regarding temperature at the time of glyphosate application are mixed. Therefore, the overall goal of this study was to evaluate tolerance to glyphosate by examining uptake and translocation of the herbicide, and to determine the influence of plant growth stage and temperature on efficacy of glyphosate in two populations of common lambsquarters collected from Kansas.

Materials and Methods

Common Lambsquarters Biotypes

Two common lambsquarters biotypes were evaluated for their susceptibility to glyphosate. In 2012, a biotype of common lambsquarters that was not controlled by glyphosate was identified in a soybean field located in north central Kansas in Dickinson County (DK). A local grower acquired the land in 2012. There was no crop grown on the land the previous year. According to the farmer, the field was overrun with weeds and 90% were common lambsquarters. The weeds were so prominent that the farmer, who normally practices no till, tilled the ground in the spring before planting as a first attempt to manage the weeds. A soybean crop was planted on the tilled field. The soybean field was then sprayed POST four times throughout the season with a field rate of glyphosate plus surfactant. After four treatments, the common lambsquarters were still prevalent in the field. It should also be noted that 2012 was an exceptionally dry year with below average precipitation. The farmer harvested one plant

representing the surviving common lambsquarters biotype and brought it to Mid Kansas Co-Op in Abilene, KS. From there it was brought to Kansas State University where it was cleaned to get seed for testing. The second biotype used in this research was a known susceptible population from a field in Riley County (RL). Three separate experiments were conducted to evaluate the tolerance of two biotypes of common lambsquarters to glyphosate: greenhouse dose-response study, shikimate accumulation assay, and glyphosate uptake and translocation study.

Glyphosate Dose-Response

Seed from the Dickinson County (DK) soybean field and the susceptible biotype from Riley County (RL) were germinated in flats (20 cm by 28 cm) filled with commercial growing media (MiracleGro®, Scotts Miracle-Gro Products, Inc., P.O. Box 606, Marysville, OH) in the greenhouse attached to the Agronomy Department at Kansas State University. The greenhouse was maintained at 25/20 C d/n and 15 h photoperiod supplemented with 200 $\mu\text{mol}/\text{m}^2/\text{s}$ PPFD provided with sodium vapor lamps. Individual plants were transplanted into commercial growing media in 6 by 6-cm pots. Glyphosate doses of 0, 105, 210, 420, 840, and 1680 g ae/ha plus 2% (v/v) ammonium sulfate (AMS) using a chamber bench-type sprayer calibrated to deliver 187 L/ha at 138 kPa on plants 8 to 10-cm tall. The experiments were conducted in a completely randomized design with six replications and then repeated. Three weeks after treatment (WAT), the dry biomass of the plants was determined and a three-parameter log-logistic regression model was fit to the biomass data:

Equation 2.1 Three parameter non-linear regression model.

$$Y = \frac{d}{\{1 + \exp(b(\log(x) - \log(e)))\}}$$

where Y is aboveground biomass (g per plant), e (also known as GR₅₀) denotes the herbicide dose that caused 50% response, d is the response upper limit, b denotes the relative slope around

e, and x represents herbicide dose (g ae/ha). The response lower limit was set equal to 0 (Seefeldt et al., 1995).

Shikimate Accumulation Assay

Shikimate accumulation assay was performed using Shaner et al. (2005) method because glyphosate-susceptible plants will accumulate shikimate after exposure to glyphosate. Fifteen and six plants that were 10 to 15-cm tall from DK and RL, respectively, were selected for the assay. Four 5-mm leaf discs were collected from the youngest fully-expanded leaf of each plant. Leaf discs were placed in a 96-well microtiter plate with one leaf disc per well. Leaf discs were placed in either a buffer solution (0.6902 g ammonium phosphate dissolved in 600 ml deionized water) or a glyphosate solution (100 μ M glyphosate). The plates were then wrapped with clear plastic wrap and incubated for 16 h under continuous light. Following the incubation period, the plates were frozen at -20 C for 20 min and thawed at 60 C for 20 min. After thawing, leaf discs were treated with 1.25 N HCl (25 μ L) and incubated at 60 C for 20 min. A new 96-well microtiter plate was filled with 25 μ L of solution from the treated leaf discs and 100 μ L of reaction buffer (periodic acid (0.25% v/v)/meta-periodate (0.25% v/v)) was added and incubated at 40 C for 20 min. Subsequently, 100 μ L of quenching buffer (0.6 M sodium hydroxide/0.22 M sodium sulfite) was added to each well. Optical density (OD_{380}) was then measured using a spectrophotometer (Epoch Micro-Volume Spectrophotometer System, BioTek, Winooski, VT) at 380 nm equipped with Gen5 version 2.01 software. A shikimate accumulation standard curve was generated and used to calculate the shikimate accumulation in each well. The values for 0 μ M glyphosate treatment were subtracted from the 100 μ M glyphosate treatment to determine the accumulation of shikimate in ng shikimate/ μ L solution. The assay was performed three times per plant (Shaner et al., 2005).

Glyphosate Uptake and Translocation

Uptake and translocation of glyphosate by the DK and RL biotypes were evaluated by uniformly treating the youngest fully-expanded leaf of 6 to 8-cm tall plants with ¹⁴C-labeled glyphosate (~ 200,000 dpm) where were then placed in a growth chamber. The treated leaf, plant parts above treated leaf, and plant parts below treated leaf were harvested at 6, 24, 48, 72, and 96 h after treatment. The treated leaves were rinsed once for 30 s with 5 ml of a wash solution containing 10% v/v ethanol and 0.5% v/v Tween 20 in a 20-ml scintillation vial. Then 10 ml of scintillation cocktail was added to vials for measuring unabsorbed radioactivity. All plant parts, including the washed treated leaves, were dried for 72 h at 65 C and stored at 40 C until oxidized. Individual plant parts were oxidized using a biological oxidizer (OX-510, RJ Harvey Instrument Corp, Tappan, NY) and evolved CO₂ was trapped in 15 ml of scintillation cocktail. Radioactivity recovered from each plant, including the rinsate, was determined using liquid scintillation spectrometry (Tricarb 2100 TR Liquid Scintillation Analyzer). The percent of herbicide absorbed was calculated as a percentage of total radioactivity applied using the equation:

Equation 2.2 Herbicide absorption.

$$\text{Herbicide absorption (\%)} = \frac{\text{Radioactivity applied} - \text{Radioactivity in rinsate}}{\text{Radioactivity applied}} \times 100$$

Translocation (distribution) of glyphosate within the plant was determined by expressing radioactivity recovered in a given plant part as percentage of the total radioactivity recovered (i.e. radioactivity applied minus radioactivity in the rinsate) using the following equation:

Equation 2.3 Herbicide translocation.

Herbicide in a given plant part (%)

$$= \frac{\text{Radioactivity recovered in a given plant part}}{\text{Radioactivity applied} - \text{Radioactivity in rinsate}} \times 100$$

Common Lambsquarters Growth Stage and Glyphosate Efficacy

Plants of RL and DK biotypes were evaluated for glyphosate efficacy at different growth stages. Individual plants were grown in 10 by 10 by 12-cm pots containing a commercial growing media. The experiment was conducted in a greenhouse maintained at 25/20 C d/n and 15 h photoperiod. Once plants reached the appropriate growth stages (5-7, 10-12, 15-17 or 19-21 cm tall), they were treated with glyphosate at a rate of 840 g ae/ha with 2% (v/v) ammonium sulfate (AMS) using a chamber bench-type sprayer calibrated to deliver 187 L/ha at 138 kPa. Experiments were conducted in a completely randomized design with four replications. Experiments were repeated. Photos were taken 1 and 2 WAT. Visual injury was estimated and recorded 1 WAT where 0 is equal to no injury and 100 is equal to no survival. Aboveground biomass (g/plant) was recorded 2 WAT. Data were analyzed using ANOVA and the means were separated with Fisher's LSD ($\alpha = 0.05$).

Common Lambsquarters Growth Temperature Effect on Glyphosate Efficacy

Plants of RL and DK biotypes were evaluated for glyphosate efficacy when grown at different temperature regimes. Individual plants were grown in a greenhouse in 10 by 10 by 12-cm pots containing a commercial growing media. When plants were 5 to 7-cm tall, they were moved to one of three growth chambers that were maintained at low (25/15 C d/n), medium (32.5/22.5 C), and high (40/30 C) temperatures with 15/9 h d/n photoperiod. After 7 to 10 days, when plants reached 8 to 10 cm, all plants were removed at the same time from all chambers. Plants were treated with glyphosate rates of 0, 105, 210, 420, 630, 840 and 1680 g ae/ha with 2%

(w/v) ammonium sulfate (AMS) using a chamber bench-type sprayer calibrated to deliver 187 L/ha at 138 kPa. Experiments were conducted in a completely randomized design with four replications and then repeated. Photos were taken 1 and 2 WAT. Visual injury data were collected 1 WAT and were analyzed using the R version 3.1.1 Model 1 with parameters set from 0 to 100% visual injury. Plants were harvested and above ground fresh weight (FW) and dry weight (DW) were recorded at 2 WAT. Data were analyzed using R version 3.1.1 Model 5 with parameters set from 0 to 100% dry biomass. Dose rates were 0 to 1680 g ae/ha.

Results

Tolerance to Glyphosate of Two Biotypes

Glyphosate Dose-Response

Results of the glyphosate dose-response study indicated nearly 20% of the DK biotype of common lambsquarters survived 1680 g ae/ha glyphosate at 3 WAT. DK biotype was more tolerant to glyphosate with a GR₅₀ of 552 g ae/ha glyphosate compared to the RL biotype with GR₅₀ of 373 g ae/ha (Figure 2.1). These results suggest the DK biotype may be more tolerant than the RL biotype; however, the DK biotype is still able to be controlled with a field rate (840 g ae/ha) of glyphosate.

Shikimate Accumulation Assay

Glyphosate inhibits production of the aromatic amino acids in the shikimic acid pathway causing a build-up of shikimate-3-phosphate, a substrate of EPSPS and its dephosphorylated state-shikimate (Shaner et al., 2005). Results for shikimate accumulation assay showed slightly less shikimate accumulation in the leaf discs of the DK biotype compared to the RL biotype. The DK and RL biotypes accumulated 14.9 ng/uL and 19.2 ng/uL of shikimate, respectively (Figure 2.2), suggesting that the DK biotype was slightly more tolerant to glyphosate. Approximately

one half of the plants from the DK biotype accumulated as much shikimate (ng/uL) as the RL plants did (Figure 2.2). Individual plants from the DK biotype showed variation in response to glyphosate, which suggests that the population was not homogeneous and may be segregating for level of tolerance to glyphosate.

Glyphosate Uptake and Translocation

Results showed minimal differences in ^{14}C -labeled glyphosate uptake or translocation between the RL and DK biotypes (Table 2.1). Overall uptake in RL biotype ranged from 9 to 22% from 6 h to 96 h and in the DK biotype ranged from 10 to 24% from 6 h to 48 h of total ^{14}C -labeled glyphosate applied, with absorption of 22% occurring at 96 h for RL and 23% at 96 h for DK. At all harvest times, most of the recovered radioactivity remained in the treated leaves of both biotypes (Table 2.1). Percent radioactivity in the treated leaves was inconsistent throughout all harvest periods with approximately 20% variation in glyphosate absorption at 6 and 48 h (68 to 88%) for the RL biotype and at 6 and 72 h (87 to 56%) for the DK biotype.

Less than 20% of applied radioactivity was recovered from plant parts above-treated leaves and less than 35% was recovered from below-treated leaves across biotypes (Table 2.1). The amounts were more consistent for the above-treated leaves for both RL and DK biotypes with increased translocation at 6 to 96 h (4 to 15%); whereas, the greatest amount of glyphosate was translocated to below-treated leaves at 6 and 96 h (28 and 15%) for RL and 6 and 72 h (10 and 34%) for DK.

Common Lambsquarters Growth Stage and Glyphosate Efficacy

Based on the means of the visual injury recordings, glyphosate was more effective on common lambsquarters when the plants were treated at early stages (5-7 and 10-12 cm tall) compared to later stages (15-17 and 19-21 cm tall) (Table 2.2). Upon treatment with 1x rate of

glyphosate, 5-7 and 10-12 cm plants showed injury symptoms of stunting and general chlorosis that were followed by slow death. At least half of the 15-17 and 19-21 cm plants survived the 1x rate of glyphosate. The plants that survived glyphosate application were stunted with some visible chlorosis, and subsequent growth was slow. At 2 WAT surviving plants showed new growth at the lower branches indicating a recovery from the glyphosate treatment (Table 2.2). In general, greater than 65 to 85% injury was recorded 2 WAT when 1x rate of glyphosate was applied to 5-7 cm plants, but injury was less than 50% when glyphosate was applied to the 19-21 cm plants. Reductions in the DW of common lambsquarters after glyphosate treatment were related to visual injury ratings (Figures 2.3, 2.4 and 2.5). Percent reduction in common lambsquarters DW as a result of glyphosate application was less in 19-21 cm plants when compared to 5-7 cm plants.

Common Lambsquarters Growth Temperature Effect on Glyphosate Efficacy

Glyphosate was more effective on common lambsquarters when grown under low temperature (25/15 C d/n) compared to medium (32.5/22.5 C) or high (40/30 C) temperatures (Figures 2.6 and 2.7). For both DK and RL biotypes, visual injury was greatest on plants grown at low temperature even at 0.125x rate of glyphosate compared to no more than 20% visual injury occurring at the 1x rate of glyphosate on plants grown under high temperature (Figure 2.6). Glyphosate rates required to reduce biomass (GR_{50}) of RL and DK biotypes 2 WAT were significantly lower for common lambsquarters plants grown at low temperatures than high temperature (Figures 2.8 and 2.9). Plants grew faster and produced more biomass when grown at lower temperatures and slower with reduced biomass at higher temperature. These results suggest that the glyphosate efficacy appeared to be reduced when common lambsquarters were grown at higher temperatures with 75% and 81% reduction in dry biomass for RL and DK

biotypes grown in lower temperatures as opposed to 43% and 17% reduction in dry biomass for RL and DK biotypes grown in higher temperatures.

Discussion

The purpose of the whole plant dose response to glyphosate study was to determine if the DK biotype of common lambsquarters was tolerant or resistant to glyphosate. Results suggest that the DK biotype was 1.5-fold more tolerant to glyphosate than RL biotype (Figure 2.1). Growth reduction of 50% biomass of DK was 552 g ae/ha which is below the 1x rate implying the biotype has not developed resistance to glyphosate and can be controlled with a field dose rate.

Shikimate accumulation assay showed the DK biotype accumulated 4.7 ng/uL less than RL biotype suggesting some tolerance. Approximately one half of the plants from DK biotype accumulated as much shikimate as the RL plants. Individual DK plants varied in response to glyphosate suggesting that the plants in the population are not homogeneous and may be segregating for level of tolerance to glyphosate.

About 20% of the applied ^{14}C -labeled glyphosate was absorbed in both populations at 48 h. The DK biotype showed significant difference in absorption from the RL biotype (Table 2.1). Yerka et al. (3013) found a greater percentage of absorbed ^{14}C -labeled glyphosate was translocated to the above-treated leaf. In this study, the highest concentration of ^{14}C glyphosate was in the treated leaf compared to the above-treated leaf and below-treated leaf plant parts. Non-linear regression models are used to estimate A_{max} , the time at which maximum absorption occurs (Kniss et al., 2007). The final harvest occurred 96 h after application in both biotypes with the majority of the ^{14}C -glyphosate remaining in the treated leaf. The only significant difference between the biotypes occurred at 48 h. Maximum absorption occurred in RL at 96 h and in DK at 48 h after treatment. Similar absorption rates between the DK and RL biotypes of

common lambsquarters suggest different absorption does not contribute to different levels of tolerance.

Plants for the uptake and translocation study were 5 to 8 cm tall. Other researchers have found greater translocation to above-treated leaf in plants 15-cm tall due to larger carbohydrate sink (Chachalis et al., 2001; Devine et al., 1993; Hennigh et al., 2005). On the other hand, Schuster et al. (2007) found more rapid translocation in 15-cm tall plants and speculated the difference could be partly due to less accumulation of glyphosate per unit plant tissue. The smaller plants in this study could account for higher carbohydrate levels in the plant cells resulting in reduced movement to above or below treated leaf.

Previous research has also found uptake and translocation can be influenced by membrane permeability to glyphosate (Wyrill & Burnside, 1977). Thickness of common lambsquarters leaf cuticle may play a role in slow absorption of glyphosate resulting in decreased absorption over time. Riechers et al. (1994) suggest that surfactant efficacy is at least partially influenced by the plant's ability to diffuse herbicide out of the cuticle into the apoplast where it alters membrane permeability to glyphosate. Overall, there was no significant difference of uptake and translocation in both biotypes so it would difficult at this point to determine if there is any tolerance in the DK biotype.

Glyphosate provides more control of smaller than larger common lambsquarters plants (Figure 2.3). Results of this study were similar to findings of Schuster et al. (2007) of increased glyphosate injury on 2.5- and 7.5-cm common lambsquarters plants compared to 15-cm plants. Similar to this research, Schuster et al. (2007) also reported new growth on lateral and basal branches of 15-cm plants surviving glyphosate at 1.1 kg ha⁻¹ application. However, Schuster et al. (2007) found variation across populations of common lambsquarters from Kansas, Nebraska,

North Dakota, and Ohio with the Ohio population showing less susceptibility to glyphosate than the other populations. One possible explanation for the Ohio population showing less susceptibility could be the fact that the Ohio field had a history of more frequent glyphosate applications which may have caused a slow accumulation of genes and alleles resulting in a small increase in glyphosate tolerance. Therefore, more research examining plant growth stage across multiple populations is needed.

Plant growth temperature experiments showed a significant difference in common lambsquarters plant height and glyphosate efficacy. Although plants at lower temperatures were taller, glyphosate efficacy was still greater at lower rates than when applied to shorter plants at higher temperatures. It is possible that metabolism is greater in faster-growing plants. Because glyphosate is a systemic herbicide, it will move throughout a faster growing plant more quickly resulting in greater efficacy. Results of previous studies examining the effect of air temperature and relative humidity on glyphosate efficacy are inconsistent, with some showing increased absorption of glyphosate with high relative humidity (eg: Adkins et al. 1998; McWhorter & Azliin, 1980), while others have found a negative relationship (Waltz et al., 2004) or no relationship (Sivesind et al., 2011). Results of this study suggest greater efficacy at lower temperatures; therefore, more research should be done to determine the effect of air temperature and glyphosate efficacy. Field studies should be done to compare results with greenhouse studies in order to determine the variability of environmental conditions on glyphosate efficacy in controlling common lambsquarters.

Overall, the combined results of this research suggest that the DK biotype of common lambsquarters found to be uncontrolled by glyphosate in a soybean field located in north central Kansas in Dickinson County exhibited elevated tolerance to glyphosate compared to the

susceptible RL biotype from Riley County. Although common lambsquarters resistance to glyphosate has not been confirmed, varying tolerance among plants brings practical problems to weed evolution. The development of glyphosate-resistant crops has led to speculation that weeds will develop a resistance more rapidly. History has shown that since the introduction of glyphosate-resistant crops, weeds have increasingly evolved to become tolerant or resistant to glyphosate. This trend will continue if reliance on glyphosate for weed control is not lessened. Crop producers should be more proactive in utilizing diverse weed management strategies. Using various tank mixes and modes of action will help slow the evolution of herbicide tolerance and resistance. Results of plant growth stage and temperature experiments found greater glyphosate efficacy on smaller plant sizes and in cooler temperatures. The DK biotype was found to be uncontrollable throughout the growing season during a hot, dry year. It is possible that the environmental conditions contributed to the elevated tolerance or possible evolution of tolerance in the plants. Repeating this research under field conditions is necessary to help determine the contributing factors to the elevated tolerance of this common lambsquarters population from Dickinson County to glyphosate.

Literature Cited

- Adkins SW, Tanpipat JT, Swarbrick T, Boersma M (1998). Influence of environmental factors on glyphosate when applied to *Avena fatua* or *Urochloa panicoides*. *Weed Research* 38:129-138
- Baylis AD (2000). Why glyphosate is a global herbicide: Strengths, weaknesses and prospects. *Pest Management Science* 56:299-308
- Beckett TH, Stoller EW, Wax LM (1988). Interference of four annual weeds in corn (*Zea mays*). *Weed Science* 36:764-769
- Chachalis D, Reddy KN, Elmore DD, Steele L (2001). Herbicide efficacy, leaf structure, and spray droplet contact angle among *Ipomoea* species and smallflower morningglory. *Weed Science* 49:628-634
- Crook TM, Renner KA (1990). Common lambsquarters (*Chenopodium album*) competition and time of removal in soybeans (*Glycine max*). *Weed Science* 38:358-364
- Curran B, Sprague C, Stachler J, Loux M (2007). Biology and management of common lambsquarters. Glyphosate, weeds, and crops. Purdue Extension Education. GWC-11
- Dall'Armellina AA, Zimdahl RL (1989). Effect of watering frequency, drought, and glyphosate on growth of field bindweed (*Convolvulus arvensis*). *Weed Science* 37:314-318
- Devine MD, Duke SO, Fedtke C (1993). *Physiology of Herbicide Action*. Englewood Cliffs, NJ: Prentice Hall Press, Pp. 95-109
- Fielding RJ, Stoller EW (1990). Effects of additives on the efficacy, uptake, and translocation of methyl ester of thifensulfuron. *Weed Science* 38:172-178

- Fuerst EP, Barrett M, Penner D (1986). Control of triazine-resistant common lambsquarters (*Chenopodium album*) and two pigweed species (*Amaranthus* spp) in corn (*Zea mays*). Weed Science 34:440-443
- Gentsch BJ, Weber JB (1984). The efficacy of bromoxynil applied in irrigation water. Southern Weed Science Society 37:112-118
- Hagood ES (1989). Control of triazine-resistant smooth pigweed (*Amaranthus hybridus*) and common lambsquarters (*Chenopodium album*) in no-till corn (*Zea mays*) Weed Technology 3:136-142
- Heap IM (2015). The international survey of herbicide resistant weeds. Online. Internet. January 15, 2015. Available www.weedscience.org
- Hennigh DS, Al-Khatib K, Stahlman PW, Shoup DE (2005). Prairie cupgrass (*Eriochloa contracta*) and windmillgrass (*Chloris verticillata*) respond to glyphosate and acetyl-CoA carboxylase-inhibiting herbicides. Weed Science 53:315-322
- Hite GA, King SR, Hagood ES, Holtzman GI (2008). Differential response of a Virginia common lambsquarters (*Chenopodium album*) collection to glyphosate. Weed Science 56:203-209
- Jaworski EG (1972). The mode of action of *N*-phosphonomethylglycine: Inhibition of aromatic amino acid biosynthesis. Journal of Agriculture and Food Chemistry 20:1195-1198
- Jordan TN (1977). Effects of temperature and relative humidity on the toxicity of glyphosate to bermudagrass (*Cynodon dactylon*). Weed Science 25:448-451
- Klevron TB, Wyse DL (1984). Effect of soil temperature and moisture on glyphosate and photoassimilate distribution in quackgrass (*Agropyron repens*). Weed Science 32:402-407

- Knezevic SZ, Streibig JC, Ritz C (2007). Utilizing R software package for dose-response studies: The concept and data analysis. *Weed Technology* 21: 840-848
- Kniss AR, Miller SD, Westra PH, Wilson RG (2007). Glyphosate susceptibility in common lambsquarters (*Chenopodium album*) is influenced by parental exposure. *Weed Science* 55:572-577
- Malik N (1990). Weed control during establishment and yield response of timothy (*Phleum pretense*). *Weed Technology* 4:598-605
- Masiunas JB, Weller SC (1988). Glyphosate activity in potato (*Solanum tuberosum*) under different temperature regimes and light levels. *Weed Science* 36:137-140
- McWhorter CG, Azlin WR (1978). Effects of environment on the toxicity of glyphosate to johnsongrass (*Sorghum halepense*) and soybean (*Glycine max*). *Weed Science* 26:605-608
- Myers MW, Curran WS, VanGessel MJ, Calvin DD, Mortensen DA, Majek BA, Karsten HD, Roth GW (2004). Predicting weed emergence for eight annual species in northeastern United States. *Weed Science* 52:913-919
- Reddy KN (2000). Factors affecting toxicity, absorption, and translocation of glyphosate in redvine (*Brunnichia ovate*). *Weed Technology* 14:457-462
- Riechers DE, Wax LM, Lieble RA, Bush DR (1994). Surfactant-increased glyphosate uptake into plasma membrane vesicles isolated from common lambsquarters leaves. *Plant Physiology* 105:1419-1425
- Schuster CL, Shoup DE, Al-Khatib K (2007) Response of common lambsquarters (*Chenopodium album*) to glyphosate as affected by growth stage. *Weed Science* 55:147-151

- Schweizer EE (1983). Common lambsquarters (*Chenopodium album*) interference in sugarbeets (*Beta vulgaris*). *Weed Science* 31:5-8
- Seefeldt SS, Jensen JE, Fuerst P (1995). Log-logistic analysis of herbicide dose-response relationship. *Weed Technology* 9:218-227
- Shaner DL, Nadler-Hassar T, Henry WB, Koger CH (2005). A rapid *in vivo* shikimate accumulation assay with excised leaf discs. *Weed Science* 53:769-774
- Shurtleff JL, Coble HD (1985). Interference of certain broadleaf weed species in soybeans (*Glycine max*). *Weed Science* 33:654-657
- Sivesind EC, Gaska JM, Jeschke MR, Boerboom CM, Stoltenberg DE (2011). Common lambsquarters response to glyphosate across environments. *Weed Technology* 25:44-50
- Stevens OA (1932). The number and weight of seeds produced by weeds. *American Journal of Botany* 19:784-794
- Waltz AL, Martin AR, Roeth FW, Lindquist JL (2004). Glyphosate efficacy on velvetleaf varies with application time of day. *Weed Technology* 18:931-939
- Westhoven AM, Kruger GR, Gerber CK, Stachler JM, Loux MM, Johnson WG (2008). Characterization of selected common lambsquarters (*Chenopodium album*) biotypes with tolerance to glyphosate. *Weed Science* 56:685-691
- [WSSA] Weed Science Society of America (1998). Herbicide resistance and herbicide tolerance defined. *Weed Technology* 12:789-790
- Wyrill JB III, Burnside OC (1976). Absorption, translocation, and metabolism of 2,4-D and glyphosate in common milkweed and hemp dogbane. *Weed Science* 24:557-566
- Yerka MK, Wiersma AT, Lindenmayer RB, Westra P, Johnson WG, de Leon N, Stoltenberg DE (2013). Reduced translocation is associated with tolerance of common lambsquarters (*Chenopodium album*) to glyphosate. *Weed Science* 61:353-360

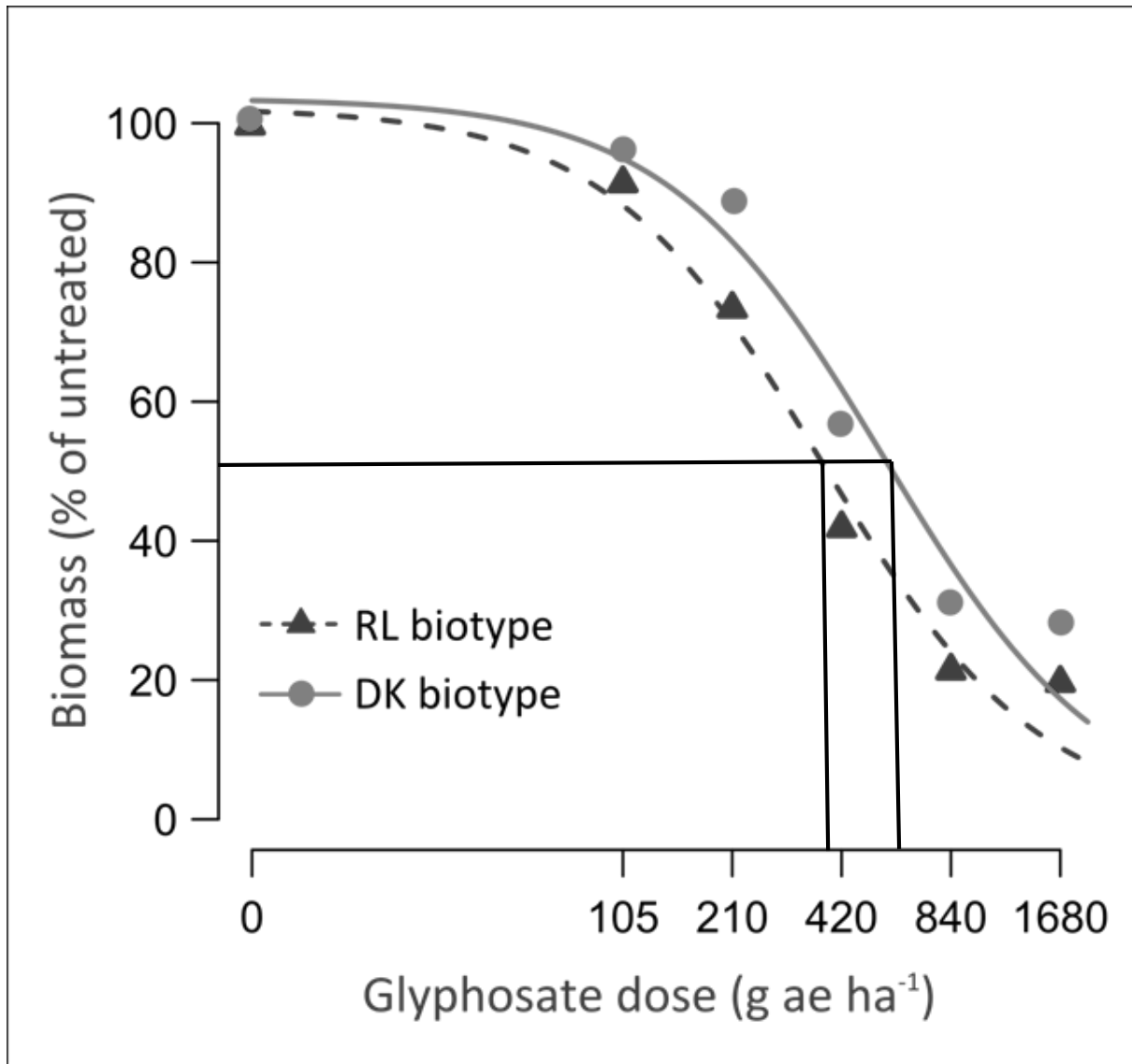


Figure 2.1. Whole-plant dose-response of DK and RL biotypes of common lambsquarters. The lines represent response curves and were generated using three-parameter log logistic regression model (Equation 2.1). GR₅₀ for DK of 552 g ae ha⁻¹ and RL of 373 g ae ha⁻¹. Nearly 20% of DK biotype survived 1680 g ae ha⁻¹ glyphosate at 3 WAT but was controlled with a field rate (840 ga ae ha⁻¹). Symbols represent means of aboveground dry biomass (% of treated).

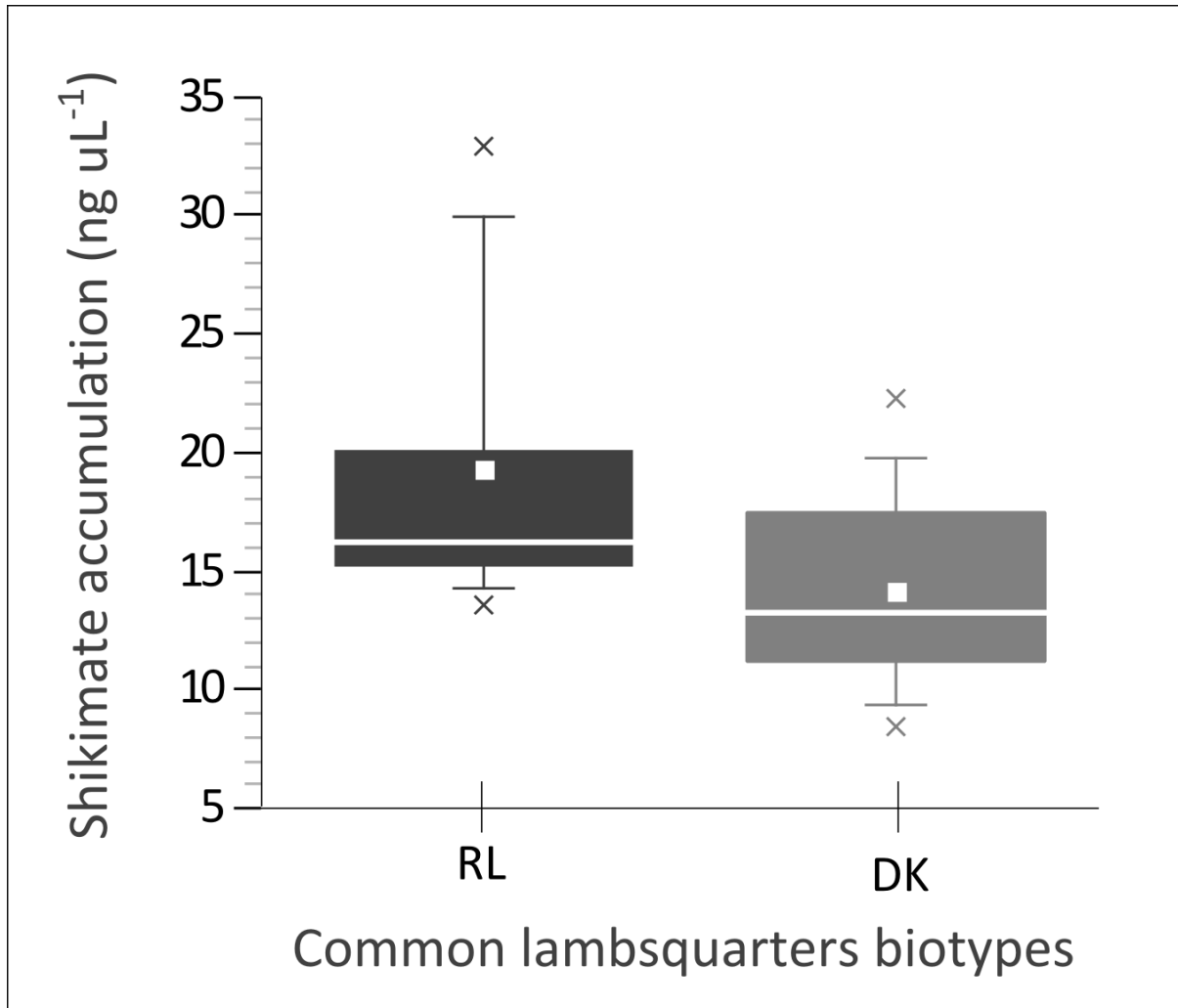


Figure 2.2. Boxplot showing shikimate accumulation (100 μM glyphosate) in RL and DK biotypes of common lambsquarters. The tails represent minimum and maximum values. The boxes represent the 50th percentile of those values. The solid line represents the median (most frequent) observation and the small square point is the mean observation. RL biotype accumulated 14.9 ng/uL of shikimate. DK biotype accumulated 19.2 ng/uL of shikimate.

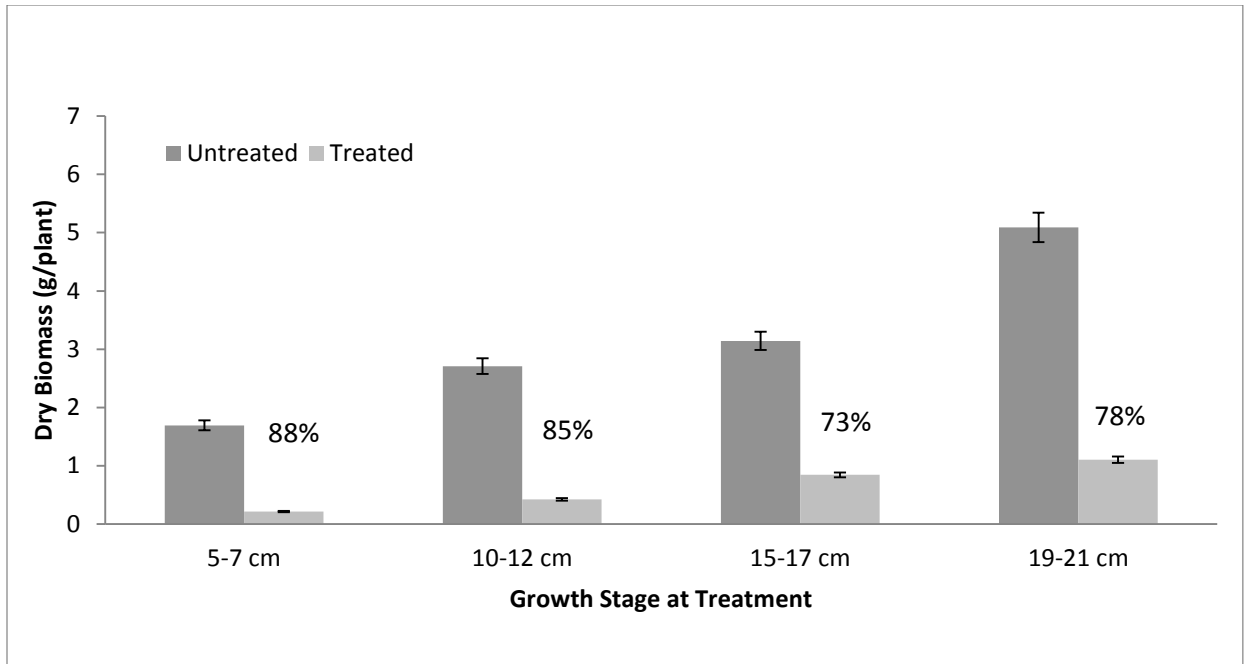


Figure 2.3. Biomass (g/plant) of RL biotype of common lambsquarters 2 WAT for treated and untreated plants. Bars represent treated and untreated plant dry weight of means. Error bars are \pm SE of two replicates. Percentages represent the difference in dry biomass between the treated and untreated plants at each plant height.

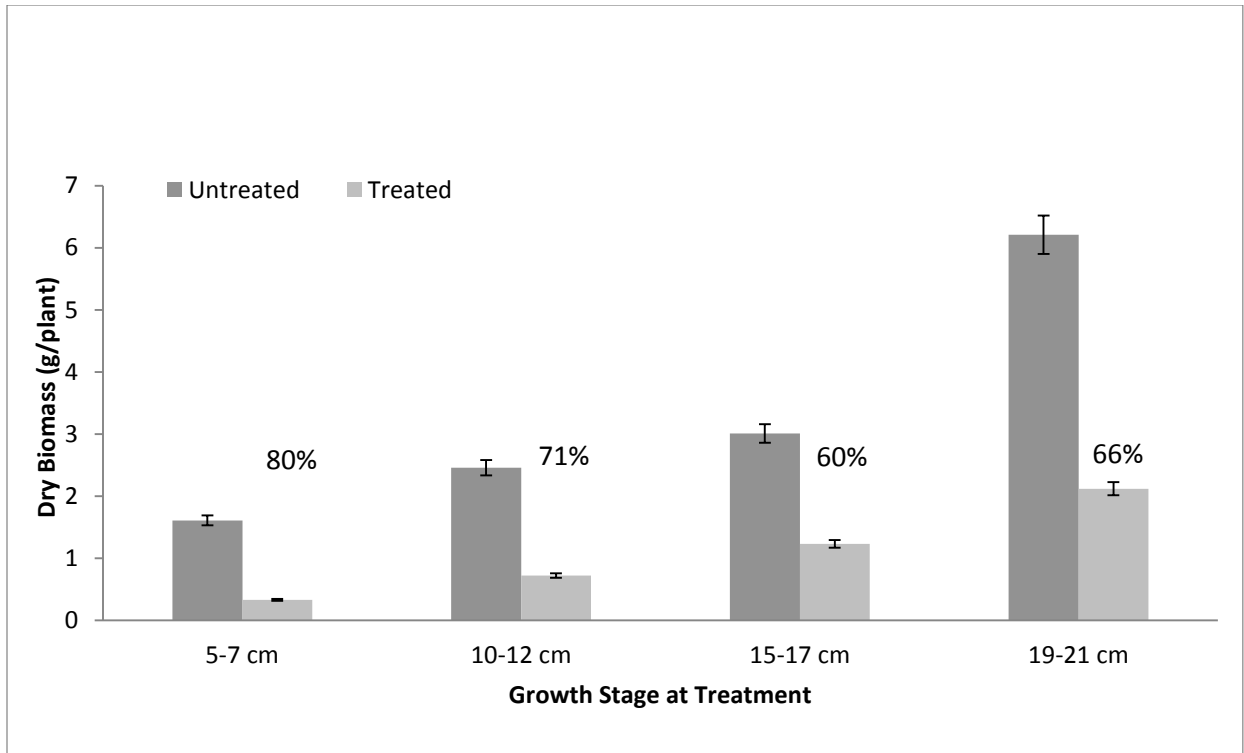


Figure 2.4. Biomass (g/plant) of DK biotype of common lambsquarters 2 WAT for treated and untreated plants. Bars represent treated and untreated plant dry weight of means. Error bars are \pm SE of two replicates. Percentages represent the difference in dry biomass between the treated and untreated plants at each plant height.

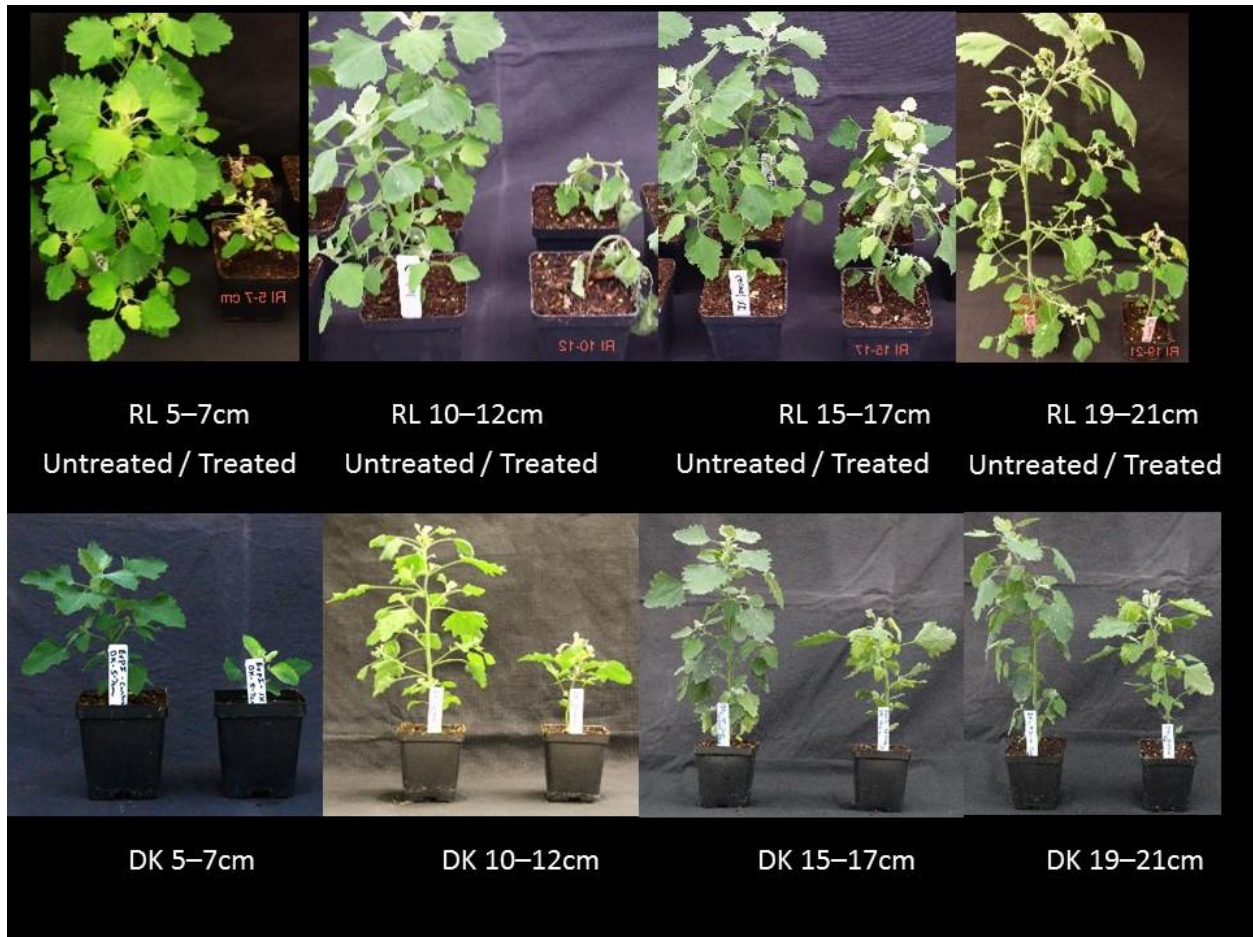


Figure 2.5. Photographs of RL (top) and DK (bottom) biotype of common lambsquarters 2 WAT untreated (left) and treated (right) with glyphosate at 1x rate.

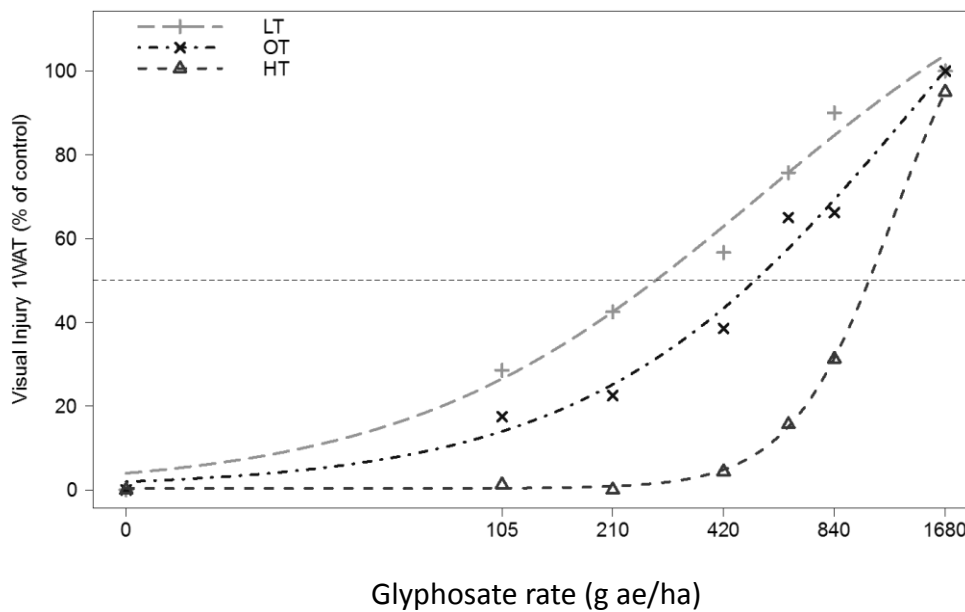
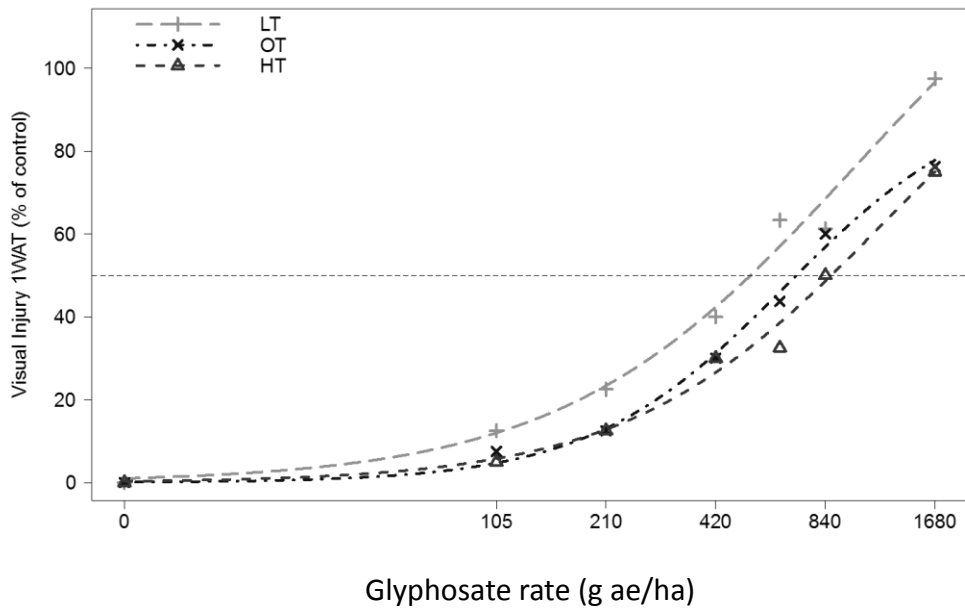


Figure 2.6. Effect of growth temperature and glyphosate efficacy visual injury RL (top) and DK (bottom) 1 WAT at day/night low (LT) 25/15 C, medium (MT) 32.5/22.5 C, and high (HT) 40/30 C temperatures. Lines represent means \pm SE of two replicates. 0% equals no injury, 100% equals plant death. Data were analyzed using R version 3.1.1 Model 1.

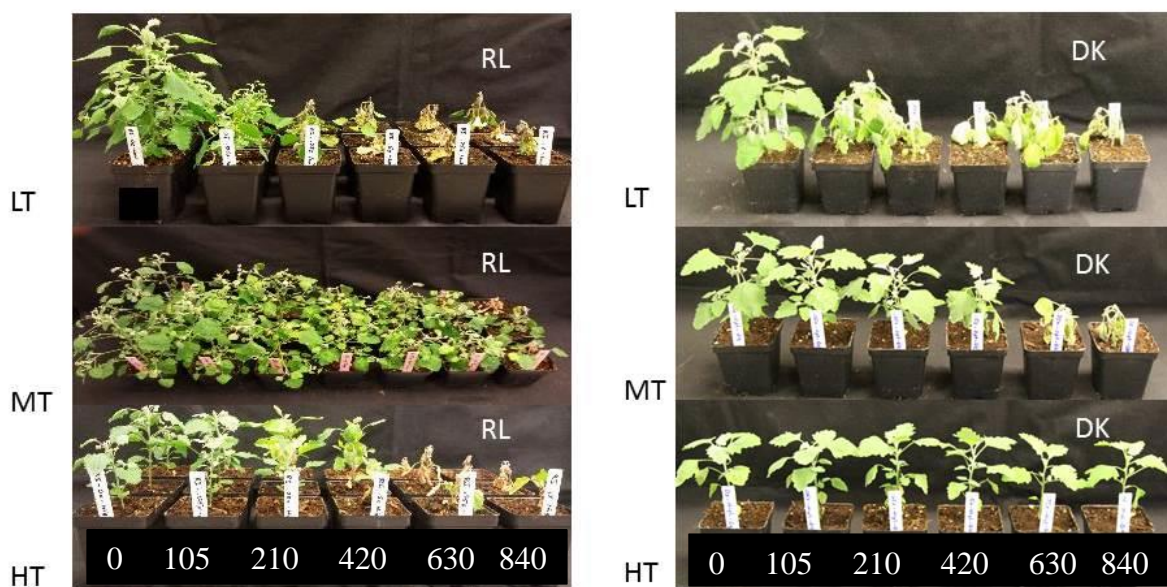


Figure 2.7. Photographs of common lambsquarters plants from RL (left) and DK (right) biotypes grown at low (LT), medium (MT), and high (HT) temperatures at 2 WAT. Plants in each photograph (left to right): untreated, 105, 210, 420, 630 and 840 g ae/ha glyphosate rates.

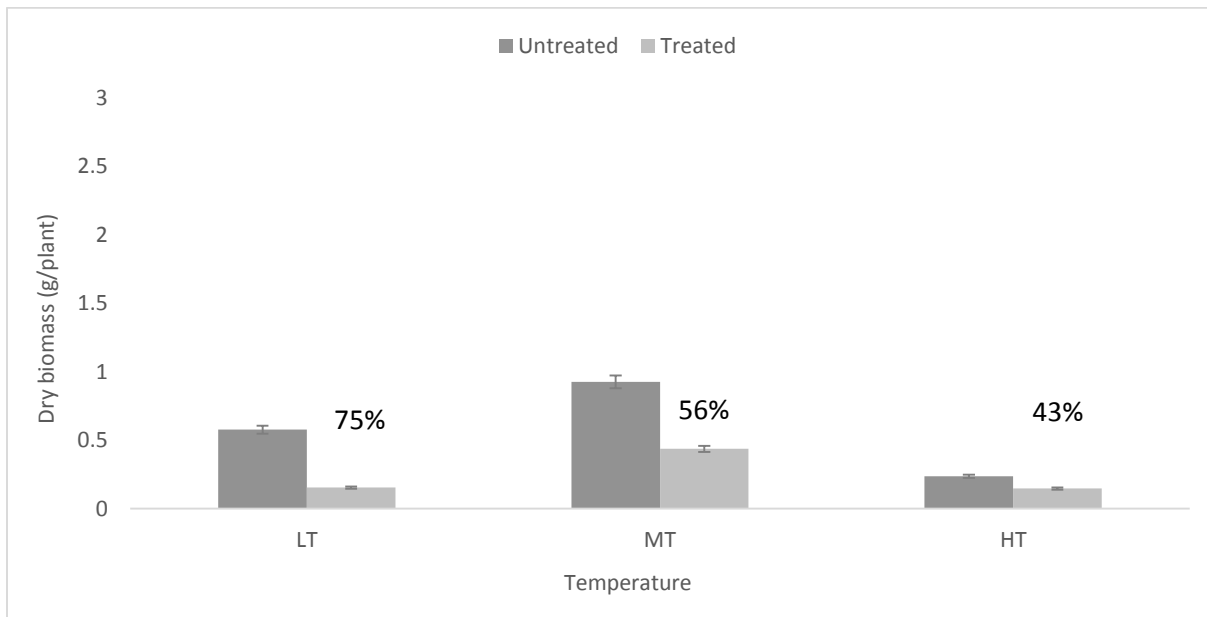


Figure 2.8. RL common lambsquarters biomass at 2 WAT in response to glyphosate as compared to untreated when grown under three temperature conditions: LT (25/15 C day/night), MT (32.5/22.5 C day/night), and HT (40/30 C day/night). Lines represent means \pm SE of two replicates. Percentages represent the difference in dry biomass between the treated and untreated plants at each plant temperature.

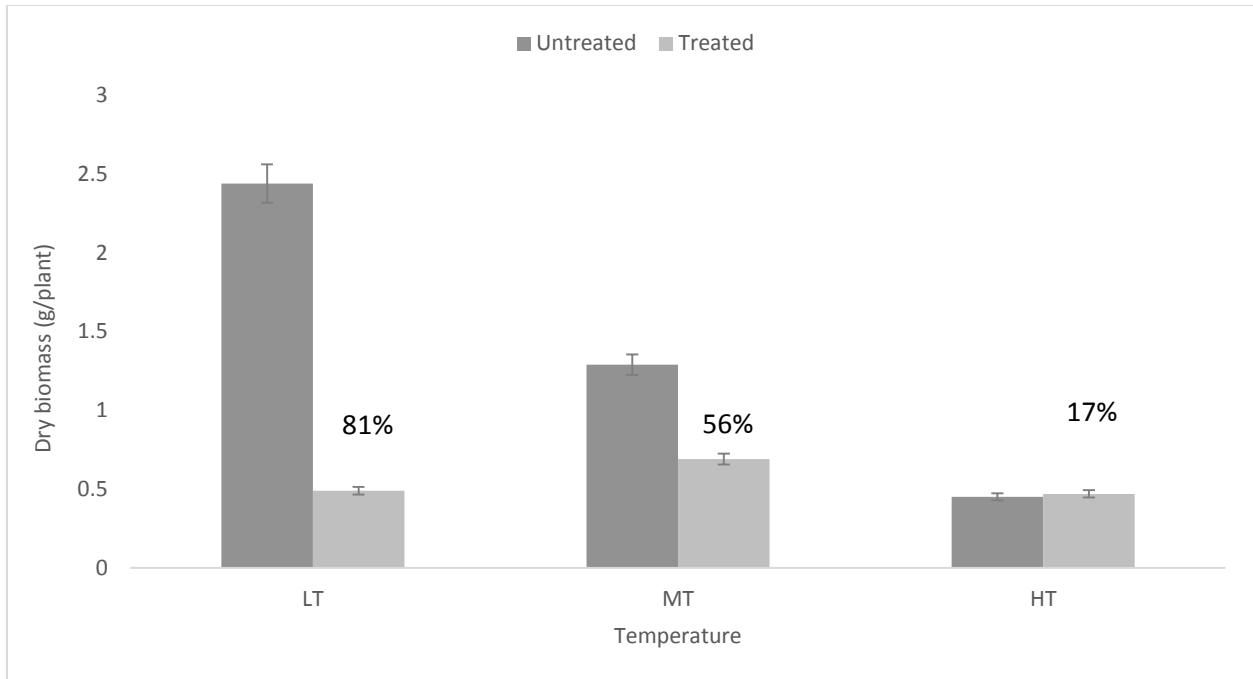


Figure 2.9. DK common lambsquarters biomass at 2 WAT in response to glyphosate as compared to untreated when grown under three temperature conditions: LT (25/15 C day/night), MT (32.5/22.5 C day/night), and HT (40/30 C day/night). Lines represent means \pm SE of two replicates. Percentages represent the difference in dry biomass between the treated and untreated plants at each plant temperature.

Table 2.1. Distribution of ¹⁴C-labeled glyphosate in RL and DK biotypes of common lambsquarters.

Plant Response	Biotype	6h	24h	48h	72h	96h
		←———— % of Applied —————→				
Absorption	RL	9	15	13*	19	22
	DK	10	19	24*	22	23
		←———— % of Absorbed —————→				
Treated Leaf	RL	68	79	88	87*	70
	DK	87	72	76	56*	75
Above Treated Leaf	RL	4	8	8	6	15
	DK	4	17	17	11	15
Below Treated Leaf	RL	28	13	4	7*	15
	DK	10	12	7	34*	10

Values are the mean of six replications. Asterisks indicate a significant difference between the DK and RL biotypes at $\alpha = 0.05$ and the absence of asterisks indicate mean values within plant parts do not differ.

Table 2.2. Combined visual injury of RL and DK biotypes of common lambsquarters for each plant height when treated 2 WAT.

Biotype	5-7 cm	10-12 cm	15-17 cm	19-21 cm
	←———— % Injury —————→			
RL	85.6 (±15.0)	67.5 (±10.4)	51.3 ^a (±3.5)	51.3 ^a (±11.3)
DK	65.0 (±5.3)	71.3 (±19.6)	33.8 ^a (±15.1)	27.5 ^a (±22.5)

Values are the mean of two replications of eight plants (±SE). (^a) indicate new growth on lower branches.